pigments

OF MICROORGANISMS

Bacteria and fungus as sources for colorants – Multidisciplinary collaboration between design and biochemistry

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Master of Arts Thesis
MA in Contemporary Design

Aalto University
School of Arts, Design and Architecture
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ABSTRACT

Colors are something that humans have always been fascinated by but impacts of different colorants have caused concern about health and environmental issues. Now more sustainable alternatives interest many industries since the demand for greener products has increased. To be able to answer these environmental challenges, biotechnology can offer new types of tools for design that are enabling co-working with living organisms as a source for new colors and material alternatives.

This research project is executed in collaboration with professionals from biochemistry and with living organisms. Research seeks after inherent natural colorants that are produced by microorganisms, bacteria and fungi. Research focuses on exploring the challenges of isolating the pigment out from the microorganisms and also considering the ethical aspects of working with living.

This research introduces a detailed description of a working procedure that was implemented with bacteria *Serratia marcescens* that is able to produce pigment called prodigiosin. In addition to this, working with another microorganism, fungi, is presented as a case study. Both of these projects focused on finding color, growing and isolating of the color from the microorganisms. To be able to tell more about the characteristics of the pigment, experimental color and material research was implemented with prodigiosin pigment that provided promising signs as a colorant.

These future colorants can offer us more environmental friendly alternatives from a natural source. Colorants that are produced by microorganisms still require more research to be done of their environmental impacts and benefits but according to the results from this research these colorants already showed potential to be scaled and further developed.

Key words

Biodesign, color, microorganism, bacteria, fungi, sustainability, prodigiosin

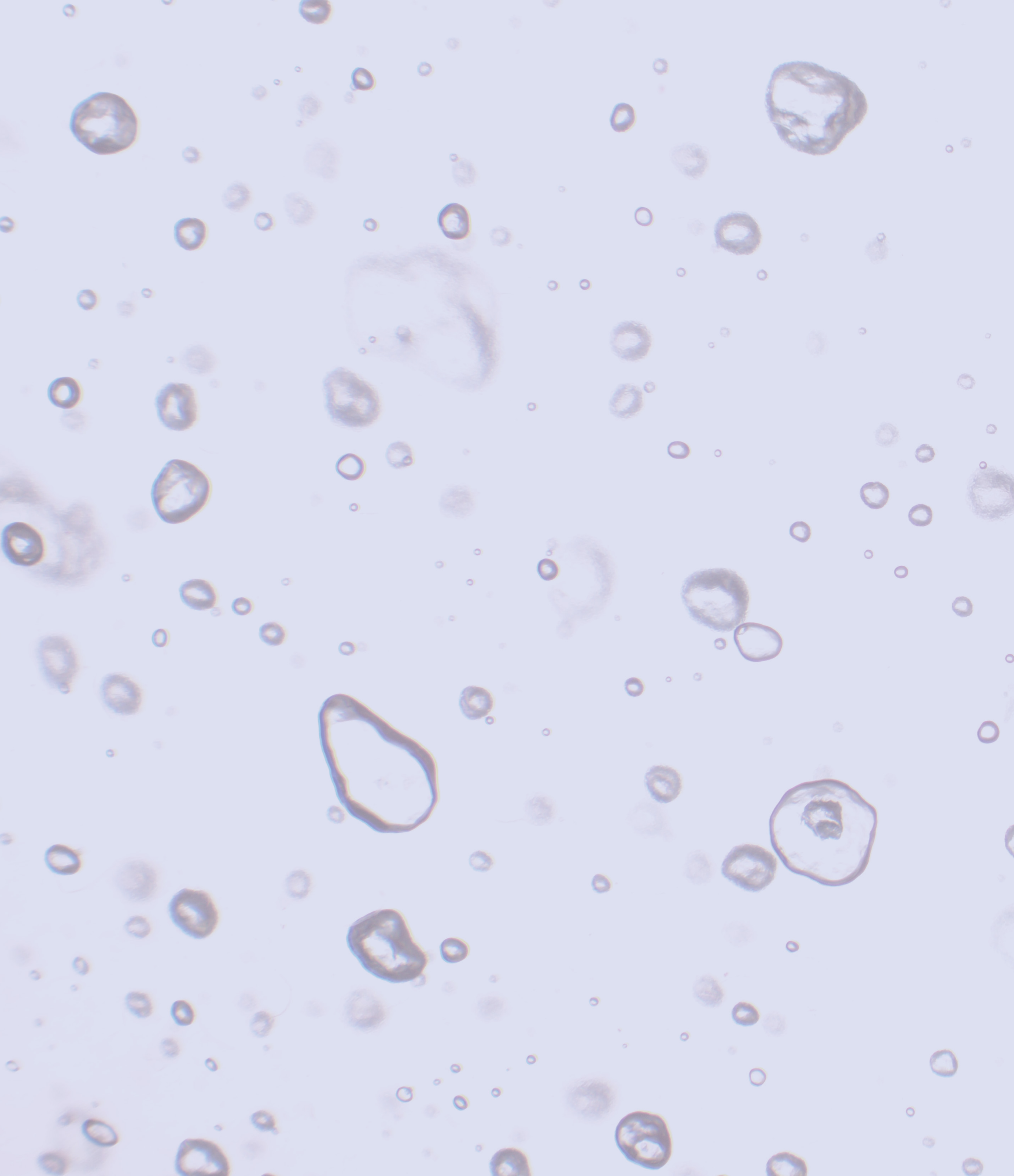

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Figure 1. Laboratory working.

1 INTRODUCTION

Design undoubtedly plays a significant role when we are solving ecological challenges in today's world. When we are looking into new innovations and problem solving I see design all around the picture as a linking tool between fields and professionals where we are solving global challenges together to build a more sustainable future. Design can have numerous roles when it comes to problem solving but how I position myself within this picture is among materials and with new innovations. We are not only challenged to create something new on a daily basis, we are here to question existing norms and standards.

Coming up with new innovative ideas is not an easy task to anyone. This is one of the reasons why I believe that we need to find the right people to help us and communicate across the fields. By challenging our own working methods and thinking we might be able to create something brilliant that was not expected in the beginning. That is why I wanted to implement this exact project to be able to challenge myself, learn, fail and to be able to feel excitement.

During my internship at Converse Inc. (Boston, U.S.) in Summer 2018 I was introduced to colorants that were produced by microorganisms for the first time. I worked at the Converse Innovation Centre and I had five different projects that were all closely related sustainability, color and material design. Already back then I was fully convinced that colors from microorganisms can be used as colorants and they could have a positive environmental impact if they are scaled into industrial production. That is why I wanted to dive deeper into the topic and learn more of the possibilities that are still simmering under the surface. Since various materials and colors have always interested me, I wanted to learn myself if we're able to produce colors with microorganisms that are in this case small living factories for brilliant colors. By understanding the working procedures and being actually in touch with microorganisms I hope to understand better the living organism and multidisciplinary work together with biochemistry professionals.

It is crucial to stay aware that color has certain characteristics as a material. It is not only something additional on top or inside of a material. By taking part of the color making process itself I wish to get closer to the materials which in this case happen to be a living organism that is creating color of its own. The aim of the project is to achieve inherent natural colorants that are produced by microorganisms, bacteria and fungi. This experimental color and material research will be executed in a laboratory environment as multidisciplinary co-working where I as a designer am learning new tools for my own design work and providing design perspective to the color development. This research was implemented together with the help of professionals from biochemistry who provided me tons of knowledge on how to work with microorganisms.

Structure of the work

In the next ***Chapter 2: Background*** I will be presenting more background for the topic and broadening the context that drives my design work. I'll be opening up my own thoughts behind my design thinking and how I implement this thinking in my own design work. I'll also share my experiences of multidisciplinary working.

Chapter 3: Research and Ethics described in detail my research question and design research method that I utilized in this project and also touched on the topic of ethics when working with living organisms.

Chapter 4: Natural Colorants and Pigments considers the terminology for these colorants and pigments. In this chapter I'll be introducing terminology that is useful while reading the text and what are the environmental impacts of current colorants. This chapter also explains what microbial colorants are and to get a deeper understanding of bacterial colorants I had an opportunity to interview Karin Fleck from Vienna Textile lab, who provided me detailed information of bacterial colorants and the current market with them.

Chapter 5: When Design Meets Science narrates the role of design within science and especially in the field of biotechnology. In this chapter I introduce terms biofabrication and biodesign that work as an umbrella for this type of design working. I'll also share my own experience of multidisciplinary working and which tools I was utilizing to find a common language together with the biochemist professionals. Within this chapter I'll present some comments considering the collaboration from my project mentors who were closely taking part of the project making.

Chapter 6: Creating Color with Microorganisms focuses on the making part where I describe the process through my working steps and by opening my own thoughts with my diary notes. First I'll describe in detail how the project started and after that move on to the making part where I introduce one procedure of making bacteria produced pigment by utilizing *Serratia marcescens* bacteria as a source of color. Within this chapter I'll also present a case study that was made in collaboration with VTT Technical Research Centre of Finland Ltd. This project is part of an ongoing BioColour project that is managed by Helsinki University and in the project we researched fungi species as sources of color.

Chapter 7: Bacterial Colorant results will present photos of implemented material experiments that were colored by using prodigiosin pigment and anso discusses about the achievements. ***Chapter 8: Discussion and Conclusions*** consists of my own reflections and learnings from the project.

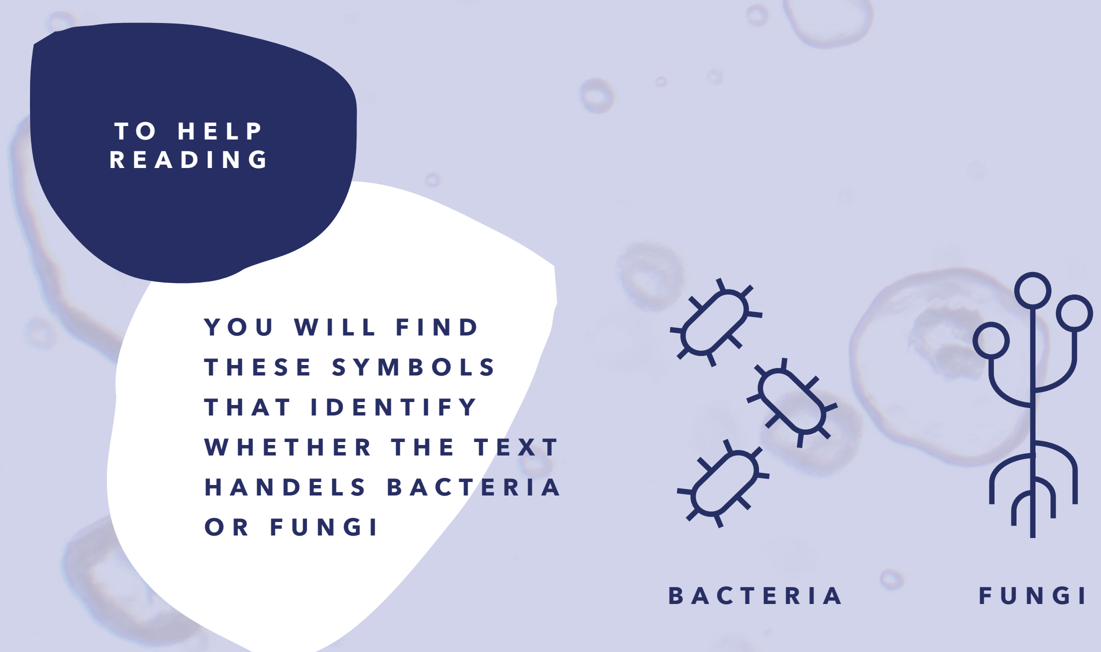


Figure 2. Symbols that help to follow the text. © Eveliina Juuri.

2 BACKGROUND

Color affects our daily life and our own color preferences are deeply attached to our emotional responses. These responses also seem to lack rational basis, yet the dominant influence of color rules our choices in our everyday life and choices from food that we eat to the clothes we wear. (Douglas Fields, 2011). We usually associate colors with aesthetics but it surely is more than that–colors can carry important information and have a significant influence on people's affect, cognition and also to behavior (Elliot & Maier, 2014). Same way as color, bacteria have a significant role in our everyday life. We wear clothes with our favourite color as our protection from sun and cold but underneath the clothes we have another protective layer on top of our skin: protective guardians, microbes. These tiny skin microbes can protect us from atrocious microbiotas that may cause diseases. In addition to that, our skin microbes also regulate our immune activation and they even produce crucial compounds that create our recognizable body scent with pheromones and allomones (Council et al., 2016).

We are living in a world where we can't always visually see all the affecting particles that have an impact in our daily life. We might find green mold in our bread as an odious blemish as the green mold is usually associated–especially in this context–as spoiled food, but at the same time we can wear a green shirt without any sensing of threat. Most of the people are disgusted and even horrified when they find out how many bacterias and other microbes live on and in us, although they have an essential role in our wellbeing. Likewise, outside of our skin and in the surrounding environment we have countless amounts of microbes that are crucial in their territory.

Microorganisms, also known as microbes, are able to produce various bioactive compounds. Microorganisms are also able to produce a spectrum of colorful pigments and these pigments can be applied into different artifacts. This may sound futuristic and relatively new, but as an phenomena this sort of color appearing is nothing new. We have evidence of this type of color occurrence already from the medieval time; there is a theory that a red-pigmented bacteria would give a naturalistic explanation to the "miraculous" appearance of blood on the Corporal of Bolsena by how the bacteria is able to produce red pigment while it grows on bread (Bennett & Bentley, 2000). Of course back then this was seen as a divine and miraculous phenomena but in this day and age, one could infer that this may have been caused by a bacteria *S. marcescens* that is able to produce red pigment, prodigiosin.

Within this thesis work I'm going to explore the laboratory working in depth with biochemistry scientists and as a designer lead the systematic experimenting as we create a color palette in collaboration with the microorganisms. The aim is to explore more sustainable alternatives for

Figure 3. Serratia marsescens growing in laboratory.

existing colorants that may contain harsh chemicals and cause water pollution among other environmental issues. This research is relevant since we are in an urgent need of more sustainable alternatives that are industrially scalable. Like summarized in *Emissions Gap Report 2019* (UNEP, 2019), it is momentous to gain a better understanding of products and the combining of sectors and plunge of materials, including implications to material quality aspect ensued from increased reuse and recycling if we are willing to decelerate climate change. This is why we have to take responsible actions while making material development and deliberate what is needed and what is actually advantageous for sustainable development.

In today's world we are facing new types of challenges while fighting against climate change. Design with a multidisciplinary approach can have an active role in tackling these challenges. Design is not only limited to aesthetics nor shaping things into new forms. The book *Design + environment: a global guide to designing greener goods* (Lewis, 2001) indicates that a designer can also have a momentous role over the environmental impacts that may arise upstream and downstream of his or her own interplay. Lewis (2001) also highlights that it is actually the product planning and design stage when the waste avoidance, water conservation, source run-down and energy efficiency can be locked into products, services and buildings.

According to the *Emissions Gap Report 2019* (UNEP, 2019) countries are extremely late in bringing out pathways to close the emissions gap, with most policies and measures so far have been inadequate. This is why we need to act urgently. Design can act a special role when it comes to sustainability and environmental impacts. Lewis (2001) pinpoints well that design for the environment can provide a unique chance to make critical interventions already in the early stage of the product development process and it can eliminate, avoid or reduce possible downstream environmental impacts. Designers among other professionals are willing to find new tools to their toolkit that can help them to discover more sustainable solutions. It is no wonder that there's an increasing number of designers who are keen on solving these critical issues by crossing fields and by exploring new material innovations.

To be able to achieve a more sustainable world I believe that we need to work interdisciplinary and try to find new innovative solutions across the fields and collaborate with different professionals. It is crucial to be aware of the environmental challenges that we're having this very day. We can act better together as a slowing buffer – as individuals but also as professionals – towards the ticking clock. This is the driving force for my thesis work and that is why I wanted to implement a multidisciplinary cooperation between design and biotechnology to find promising future material solutions with *living microorganisms*.

Figure 4. Bacteria called Janthinobacterium lividum uses its purple pigment as protection against other microbes. Photo © Artis Microbia.

2.1 WHO AM I AS A DESIGNER?

During my bachelor and master studies I have realized that designers need to consider all the consequences that designs might create. What are the sustainability challenges that we need to re-think or solve? Every designer needs to be aware of the environmental challenges that we are facing and also help with their own decisions to move consumers behavior, producers and industries into a more sustainable direction. After searching for a while for my ”own thing” in the design field, I found the field of materials and I instantly saw it as a prominent base for new innovations. Possibilities with new materials are unlimited and we have so much to explore in the world of new alternative materials. From that point I have gathered experience and practice based know-how about different materials. It was crystal clear that for my thesis work I would make a project that relates closely to materials and since I had a chance to do whatever I wanted, so I encouraged myself to try out something totally new and decided to jump into the fascinating world of biochemistry.

I’ve always valued heritable and timeless design when it serves sustainability. At its best design is something that one can cherish for decades and it lasts from generation to generation. Another feature of a good design is that it can be recycled or disposed of responsibly if one doesn’t cherish it anymore. In this matter the material always serves the purpose.

In my own design work I always keep the material in the focus and how it serves the purpose of the design. I constantly look for new, more sustainable material options and that is why I’m also keen on experimenting with new materials. Because of this passion of mine, I have done multiple material research projects and also worked together with researchers and chemical engineers with material development. Multidisciplinary working is something that I highly esteem and I find that as the most fruitful way of working.

2.2 MULTIDISCIPLINARY WORKING

As designers we have a capability to work as a bridge between different fields and industries and link them together. In combination with other disciplines, the designer emerges as a critical player in ensuring that diverse and sometimes conflicting issues are successfully built into a product. Lewis (2001) also remarks that designers’ role is limited in environmental issues when working alone. Fruitful interdisciplinary work can achieve inestimable advantages that serve as a base for great new innovations. Today’s world offers great new technologies from all disciplines that can support new solutions and that is why I see a great opportunity in multidisciplinary working while we aim for new innovations.

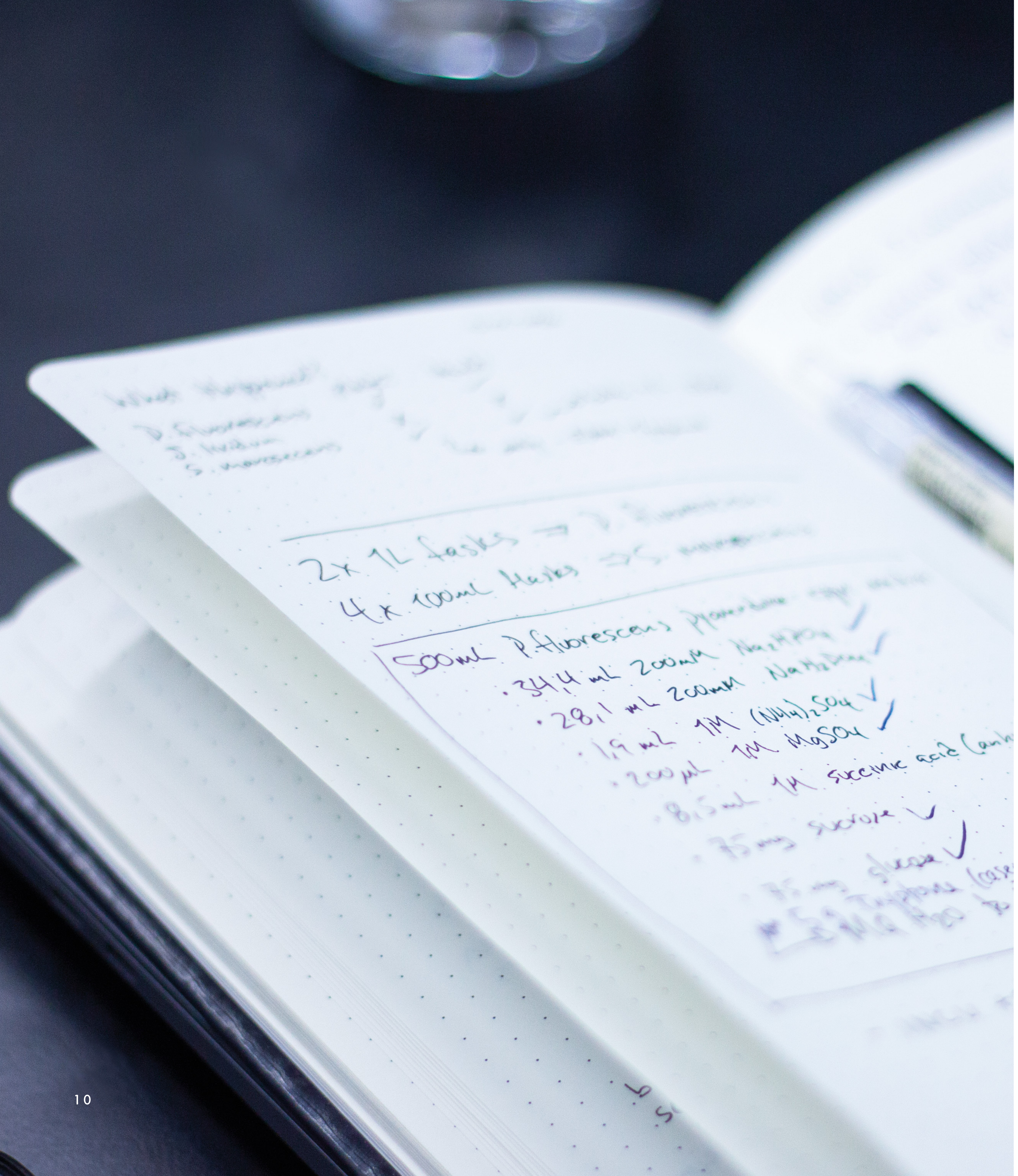
I have already gained some experience of laboratory working from my previous interdisciplinary projects so that laboratory as an environment was not totally new to me. These previous experiences form Aalto University’s CHEMARTS courses, my internship at VTT Technical Research Centre of Finland Ltd and other material development projects made within this topic area, are helping me today to make a more successful project with the professionals from the biochemistry side.

One great experience of a multidisciplinary research project was implemented together with these experts with a wood chemistry background (Fig. 5). I was collaborating together with Sanna-Liisa Järvelä and Jinze Dou and together we developed a relizable product *Kaarni* (Fig. 6) which is a biodegradable plate made out of willow bark fibers. This project started from a CHEMARTS course and we ended up to build the concept further and even won a second prize in a national contest called ’*Wood U Make it Happen?*’, a contest looking for new sustainable innovations for the wood industry.

It can not be highlighted enough that these sort of projects are fully dependent on these professionals where we as a team are developing the ideas into higher level. With only a design background I don’t really have a deep understanding of chemistry nor biology but together we can create something that wouldn’t necessarily happen without one another.

Within my thesis project, I want to break box frames between fields and prove how much more we can do when we don’t limit ourselves inside just one field as designers. As a designer I have a huge urge to always learn something new and multidisciplinary working has always inspired me throughout my studies. One key element is that I just have to find the right people who do know better than me and that is one of my core messages to all the designer peers out there: find the right people who can help you to move forward. It’s ok that you don’t understand everything, it is ok to ask stupid questions and it’s totally fine to fail more than once. Once you’ve done it, you might see that you were able to do something sticking together.

Figure 5. Our team Kaarni after contest ceremony.
Figure 6. Picture of Kaarni plate.



3 RESEARCH AND ETHICS

3.1 RESEARCH QUESTION

For my research I have selected one question which is considering the colorant making part where we're creating colorants in collaboration together with biotechnical professionals by using living microorganisms:

> ***Are we able to isolate usable color pigments out of microorganisms and what are the challenges of making this?***

This research will be implemented by utilizing microorganisms as a source for color. This means that the main focus will be in finding color but at the same time I'll be exploring what are the challenges related to the process. Because of the nature of the project, it will be executed as a multidisciplinary work. Possible challenges created from the collaboration will be evaluated by the author but also by the professionals who provided their voice for describing the challenges from their side as well.

In the following chapter methods that were used for the research making will be described more detailed.

Figure 7. Laboratory notebook.

3.2 RESEARCH METHODS

As my design research method I'm utilizing the double diamond methodology that was adapted from the Design Council (2019) article *What is the framework for innovation? Design Council's evolved Double Diamond*. The aim of the project is to understand the microorganism produced color as a material and that way to be able to answer the research question. For this reason I have created a process to follow. Figure 8 shows how this research project consists of three main sections: understanding the background, understanding the colorant making and understanding the characteristics of the colorant. These three sections build the researched topic. Each section consists of working steps that will be described later in the text. These working steps have different purposes that *discover, define*, *develop* and *deliver* the project. Based on this I created a triple diamond method for serving this design process (Fig. 9).

Each of these three main sections from Figure 8 are inside three different diamonds (Fig. 9) where the middle diamond consists of two diamonds. This design process will be implemented with each microorganism species and they can be operated simultaneously.

The first diamond of the method describes the discovering and defining by making the background research of the microorganisms and narrowing down again the possible option by selecting one microorganism for producing color. In the middle diamond we develop and deliver the colorant and help us to understand the color making. This diamond consists of two diamonds where in the middle can be evaluated if the color is fulfilling expectations as a color. The first middle diamond contains the growing, where we can try multiple methods for finding the best growing method. Based on these growings and results from them evaluate the results and decide whether to continue forward to the color

Figure 8. Understanding the Color as a Material: Created by Living Microorganisms. © Eveliina Juuri.

isolation or not. In the color isolation we again can implement various methods for the color isolation but after finding the best method for color isolation we implement a purification protocol and as a result get a purified colorant.

To get a better understanding of the colorant we will be executing experiments with different materials and with those results we have a well working selection of colored materials that work as samples and tell us if the colorant is usable in different materials. This will also deliver an answer to the research question.

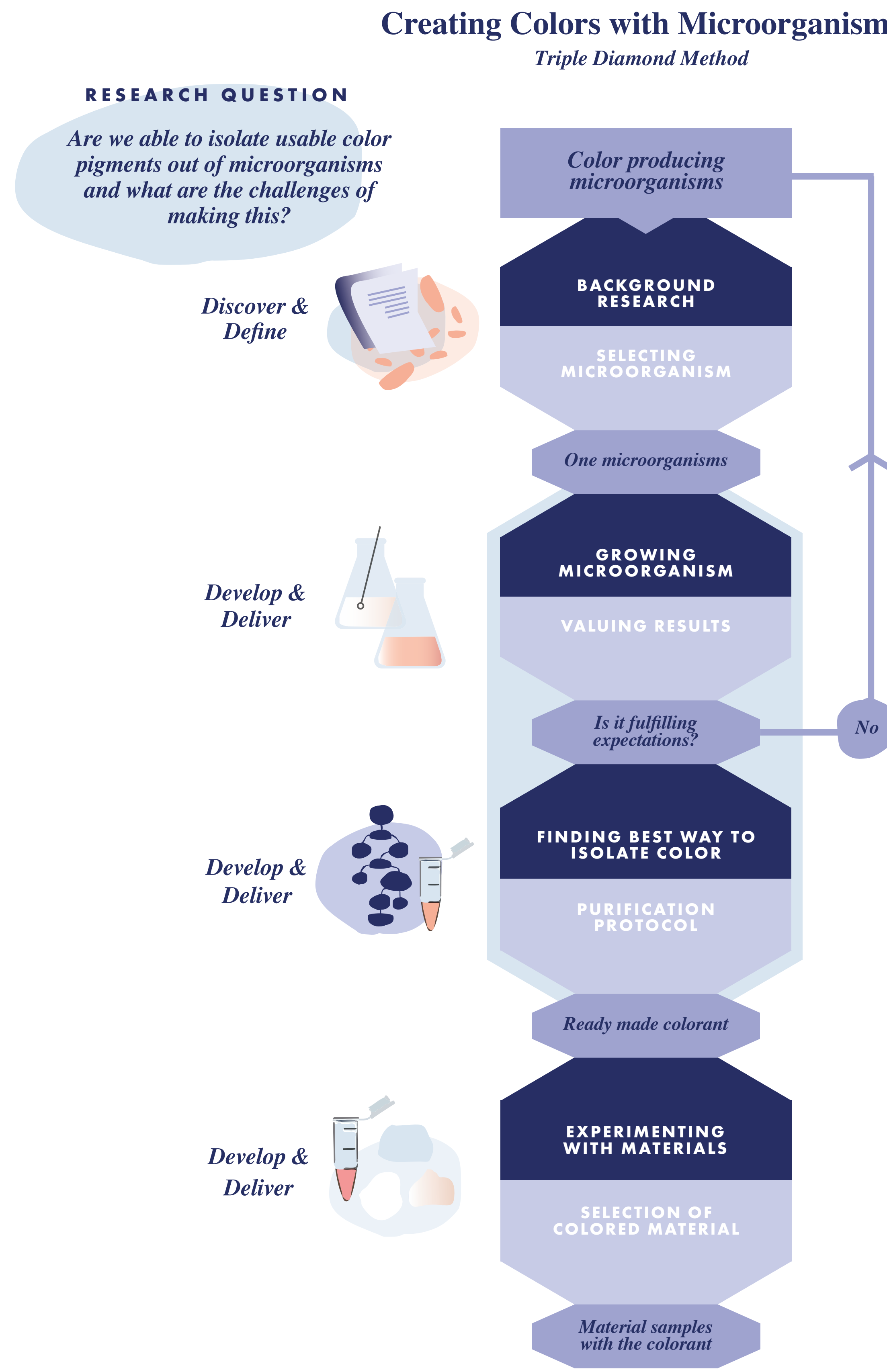


Figure 9. Creating Colors with Microorganisms: Triple Diamond Methodology. Adaptation of the Double Diamond Methodology, figure from © Design Council (2019). Figure adapted and visualized by © Eveliina Juuri.

3.3 ETHICS OF WORKING WITH LIVING

Within this project I'll be closely working with living organisms; bacteria and fungi. One of the things that I want to address is the ethics of working with the living. I wanted to touchpoint the topic since living organisms are conventional in the field of design and because these organisms are alive while we work with them, we need to have a good understanding what we are dealing with.

One way of looking at this aspect is through the chart (Collet, 2016) that is made for frameworking the design work with the living (Fig. 10). Collet (2016) frames the hierarchy into four different stages: *nature as a model, nature as a co-worker, nature as a 'hackable' system* and *nature as a 'conceptualized' system*. This chart is for framing and evaluating critically the ethical aspects while working with living. Each of the stages represent a certain level of working with living and under each category is listed key principles for each stage.

Within this chart *"Nature as a model"* represents a stage where the designer is mimicking nature's behavior, structures or function by using conventional fabrication methods. *"Nature as a co-worker"* stands for combining the mimicking of nature and cultivating techniques, meaning a stage where the designer is a cultivator who controls the shape-language by collaborating with the natural organisms, like with microorganisms. Whereas *"Nature as a 'hackable' system"* stands for tailoring the living organisms with genetic engineering where the designer acts as a 'designer biologist'. This means that biotechnology enables possibilities where organisms can be programmed to produce desired things. The last category *"Nature as a 'conceptualized' system"* represents speculative and critical design where designers can turn scientific research into more tangible scenarios and act as a provocative voice by bringing up ethical concerns and new perspectives.

Design & Living Systems Lab
Framework for Biodesign

NATURE AS A MODEL	NATURE AS A CO-WORKER	NATURE AS A 'HACKABLE' SYSTEM	NATURE AS A 'CONSEPTUALIZED' SYSTEM
BIOMIMICRY PRINCIPLES	HUSBANDRY PRINCIPLES	BIOENGINEERING PRINCIPLES	CRITICAL AND SPECULATIVE DESIGN PRINCIPLES
'NATURAL' NATURE	'NATURAL' NATURE	'SYNTHETIC' NATURE	'CONSEPTUALIZED' NATURE
DESIGNER	DESIGNER CULTIVATOR	DESIGNER BIOLOGIST	DESIGNER CRITIQUE

Figure 10. Adaptation of the "Design & Living Systems Lab: Framework for Biodesign" figure by Carole Collet, Design & Living Systems Lab (2016). Adapted from "Design & Living Systems: Selected Works" by C. Collet (2016: 116).

By looking at this chart it is easy to position this thesis project into the *"Nature as a co-worker"* category where my role as a designer is to cultivate the living organism to produce color that is delivered by the bacteria and fungi. Of course this raises questions: am I actually co-working with the bacteria or am I depriving something away from the microorganism? Are they enjoying while producing color pigments or are they stressed? Am I doing harm for nature by doing this? This is something that the scientific community speculates but we can't yet tell why certain microorganisms are producing color while they are growing.

In a way our work with the microorganisms is co-working with nature since I'm providing food for the microorganisms and they are providing us color. It is common to utilize microorganisms as part of our daily life, sometimes even without realizing that. For instance with kombucha making or while making bread with sourdough. The only difference is that we are implementing the growing in a laboratory environment for the sake of these microorganisms so that they can grow in a sterile environment where other microorganisms are not competing against them.

Microorganisms are living things and they need to be taken care of constantly to make sure that they are capable of producing color. This can also create an emotional bond between human and microorganisms, which in my mind is comparable to taking care of houseplants. It is good to keep in mind that we are not modifying the microorganisms, we are not forcing them to produce anything, we just hope that they are doing their thing by providing them proper food for living and a suitable environment. In other words, we keep nature in its natural form while cultivating it to achieve colorants which is in my mind ethically acceptable.

Figure 11. Fungal cultures.

4 NATURAL COLORANTS

4.1 COLORANTS AND PIGMENTS

Colors are something that humans have always been fascinated by. Throughout history humans have been using different sources of colors and colors have always acted an essential role in the formation of different cultures of human beings around the world (Mohd, 2016). *'Color'* as a term describes at least three various matters in our reality: subscribing to a property of an object, absorbance of light rays and determining a class of sensation in the brain (Nassau, 1997). Then again the word *'colorant'* is a common term for describing any substance that is used to transform the color of an object by changing its spectral transmittance or of by modifying its spectral reflectance (Malacara, 2011). Different origins for natural colorants are shown in Figure 12.

Colorants can be used in various industries and with different applications, for instance to color textiles, plastics, paints and prints. These colorants can be either dyes or pigments but technically speaking, distinction between these two terms is somewhat ambiguous (Kuehni 2011). Sources for natural colorants such as *dyes* or *pigments* are plants, different microbial sources, minerals and animals (Butola, 2018).

According to Malacara (2011) the difference between dyes and pigments is that dyes are soluble in the host material, typically water, while pigments are not. Although, it is important to note that pigments can be solvable with some organic solvents. When defining natural pigments in biological systems, we talk about pigments that are synthesized and accumulated in, or excreted from, living cells (Hendry 1996).

As one may notice, the terminology within this matter may appear sometimes complex and even unclear. It is important to note that within this thesis work terminology leans closely towards biology since we operate inside of the field of biochemistry. Next to this chapter I clarify a list of terms that are essential while reading this thesis work.

Figure 12. Natural colorants according to their origin. Adaptation of the figure 2.2 (Butola, 2018:24).

4.2 TERMINOLOGY

Bacterium, noun bacteria: "A member of a large group of unicellular microorganisms which have cell walls but lack organelles and an organized nucleus, including some that can cause disease" (Bacterium, 2020).

Bacterial: "Relating to or caused by bacteria" (Bacterial, 2020).

Biochemistry: "The branch of science concerned with the chemical and physico-chemical processes and substances that occur within living organisms" (Biochemistry, 2020).

Biodegradable: "(Of a substance or object) capable of being decomposed by bacteria or other living organisms and thereby avoiding pollution" (Biodegradable, 2020).

Colorant: "A substance used for coloring a material" (Colorant, 2020).

Culture: "The cultivation of bacteria, tissue cells, etc. in an artificial medium containing nutrients" (Culture, 2020).

Dye: "A natural or synthetic substance used to add a colour to or change the colour of something" (Dye, 2020).

LCA: "Life cycle assessment (LCA) is a "cradle-to-grave" approach for assessing industrial systems. "Cradle-to-grave" begins with the gathering of raw materials from the earth to create the product and ends at the point when all materials are returned to the earth" (Scientific Applications International Corporation & Curran, 2006).

Microorganism: "Microorganisms is a broad term used to encompass bacteria, yeast, fungi, and in some definition viruses" (Batt, 2016).

Organic solvent: "Organic solvents are carbon-based substances capable of dissolving or dispersing one or more other substances" (CDC, 2020). For instance acetone and ethanol.

Pigment: "A substance that imparts black or white or a color to other materials" (Pigment, 2020).

4.3 ENVIRONMENTAL IMPACTS OF COLORANTS

Multiple aspects affect how environmentally friendly colorants tend to be. Colorants are not usually used as such and often the process of coloring or dyeing may also require some other processing steps and the combination of all of these processing steps may have an impact on the environment. That is why it's important to look at the overall picture while estimating the environmental impacts of natural colorants. One way of valuing environmental impacts can happen through LCA (life cycle assessment) analysis.

Different industries like plastic, paint, paper, printing and textile industries have a great demand for coloring agents (Tuli et al., 2015). When we think about the environmental impacts of colorants, it is almost impossible to avoid discussion of the pollution caused by the fast-fashion industry. Conspicuous leaks of colored wastewater in streams and rivers of course seem worrying because of the rapid change in nature that is visible to the human eye. In textile manufacturing processes many chemicals are used for instance in the wet processing (Madhav et al., 2018). Wet processing may include bleaching, surfactants, softeners, dyestuffs, antifoaming agents, among others (Niinimäki et al., 2020) that can contain chemical compounds. These chemical compounds also have manufacturing processes that have a certain environmental impact.

This same applies also with other industries as well: if these processing parts contain lots of harmful or toxic compounds– and these compounds end up in nature or if they affect human health–they can be considered as harmful for the environment and for human health. For instance in the paint industry many metal pigments that are used in paints (e.g cadmium) are highly toxic and also non-renewable resources (Architecture & Design, 2007).

There is an increased demand from the consumer side especially towards textile industries for more natural colorants that are environmentally friendly (Mohd et al., 2017). Natural dyes have increased interest because of their biodegradability and low-toxicity level (Shen et al., 2014). Even when a consumer would be willing to pay a higher price for a "green" product, it is difficult to tell if the purchase decision is environmentally responsible (Chen & Burns, 2006). To be able to offer consumers more environmentally friendly options, it is crucial to bring new sustainable and competitive alternatives – also colorants – to the industrial use. It's good to keep in mind that by switching colorant ingredients into natural ones does not guarantee sustainability: the production process including other additives also needs to support sustainability.

Environmental arguments are oftentimes emotive and it's highly important to consider all the necessary aspects that are involved within the subject. One should not lose the focus with facts when we're estimating the environmental aspects. There is not always a clear answer whether something is an environmentally friendly alternative especially when we inspect the overall picture behind a product. This is why it is always good to question the environmental impacts of the new innovations as well.

Figure 13. Residents along Tullahan river (Philippines) have noted a multi-coloured effluent in the river water, rocks and banks. Several industries, such as paper, pen and dye factories, are located upstream from this site. Photo © Gigie Cruz-Sy / Greenpeace.

4.4 BACTERIA COLORANTS

Microorganisms, especially by fungi and bacteria, are able to produce naturally colored microbial compounds (Tirumale & Wani, 2018). These compounds can be utilized in colorants. Microorganisms are able to produce bio-pigments and those pigments can hold multiple advantages, for instance availability, cost, efficiency, high yield and easy downstream processing (Joshi, 2003). These renewable natural sources are also well suitable for bigger scale production, when compared with vegetal or animal extracts although the stability of these biopigments is less stable when compared with synthetic ones (De Carvalho et al, 2014). In addition to this, studies have shown that bacterial produced pigments can hold antioxidant, anticancer, antimicrobial (Tuli et al., 2015) and immunosuppressive activity (D'Alessio et al., 2000).

To get a better understanding of bacterial colorants and the field around the topic I had an opportunity to have a discussion together with Karin Fleck, the CEO of Vienna Textile Lab. As a company Vienna Textile Lab has a mission to match technology and design by fabricating organic colors that are made by naturally occuring bacteria (Fig. 14) according to the company's website.

Fleck has broad practice-based knowledge of the colorant business and our immersive discussion gave me lots of new thoughts and insights from this field. In the following fragments I'll sum some parts from our discussion with Fleck (2020).

What are the advantages of microorganism produced colorants?

If we compare microorganism produced dyes with plant based dyes, the field with natural dyes is quite limited. They require a lot of growing water, they take a lot of archiculture surface, they need to be harvested and these matters make them more expensive. Synthetic dyes require crude oils and chemicals, and that is why they are also depending on chemical suppliers. The ingredients for these chemicals are produced in certain areas which makes the dye producers really dependent on those chemical suppliers. The advantages of microorganism produced dyes are that they can be produced everywhere around the world for instance in fermentors. This makes them accessible, independent from crude oils, indexable and feasible as colorants. (Fleck, 2020).

Bacteria based colorants have also some other benefits: "I don't know any plant in nature that is able to produce violacein, I think it's quite unique" Fleck (2020) describes. Violacein is a violet pigment that is mainly isolated from bacteria of the genus Chromobacterium (Durán, 2007). The range of colors, possible to be produced with bacteria, is relatively broad. "Some of the color molecules that we are able to extract out from bacteria are already possible to get from plants and some of them even synthetically" Fleck (2020) explains.

Figure 14. Bacterial colorants by Vienna Textile Lab.
Photo © Vienna Textile Lab.

Are there any weak points of microorganism produced colorants?

One of the weak points of biochemistry is that most of the things are happening in water. Fleck (2020) mentions that if you're able to keep the water out of the process, you're already much more relevant with your colorant. One of the challenges is to be able to meet the color standards with the colorants that are produced with living organisms. Many textile industries and fashion houses are using Pantone LLC company's PANTONE MATCHING SYSTEM®, that is a standardized color reproduction system (Pantone, 2020), or some other color systems. With natural sources of color this creates an issue since it's hard to match the color standards perfectly (Fleck, 2020). Color stability also plays an important role. "If the bacterial dyes can not reach the dyeing quality of synthetic dyes, they are off, it's not going to be a relevant market player" Fleck (2020) explains.

Bacterial dyes also need to pass these standards that are set up by the EU government and testing for that takes a lot of effort and money. "Majority of the market doesn't want the colors to fade out, even though some synthetic dyes wash-out eventually too" Fleck (2020) mentions about the standards that bacterial colorants should reach. Oftentimes bacteria produces multiple compounds and when colors are created with bacteria, usually the color is a mixture of color molecules. When we isolate color out from bacteria it is hard to make totally pure compounds and that again makes it hard to meet the standards (Fleck, 2020). Vienna Textile Lab follows ISO (International Organization for Standardization) standards for textile testing.

I asked Fleck if they utilize bacteria produced colorants while the bacteria is still in the dyeing liquid or do they isolate the color molecules out from the bacteria. Fleck (2020) replied that they do both: when the bacteria is still in the dyeing liquid the color result is more homogenous and the process is faster. Fleck (2020) mentioned that when they implement dyeing in larger quantities they need to do the color extraction but they don't purify them into completely pure compounds if it's not necessary to meet the strict color standards.

Textile market is of course a mass market but has Vienna Textile Lab ever gotten any request of producing colorants for other purposes? "Lot of requests with printing and 3D-printing because that's hip right now" Fleck (2020) says. However Fleck mentions that if they're not funded as a company to do other uses, they rather continue with textiles (Fig. 15).

What are the environmental aspects of bacterial dyes?

Fleck (2020) mentions that understanding the different production systems our world is based on is crucial – petrochemicals and fossil originals are on the base of many industries. Fleck (2020) points out that they are not here as a company to kick natural colors out of the system. They are here to challenge synthetic colors, because 99% world wide color production is synthetic. "I think creating an alternative that is available in the market is important" Fleck (2020) notices. However, Fleck believes that it is not possible to beat synthetic dyes totally. "These whole bacterial dyes will be successful if you find for instance new colors -- if you are able to find new color molecules that haven't been known for the industry that have new properties" Fleck (2020) states about the potentiality of bacteria produced colorants.

As mentioned in *Chapter 4.3 Environmental impacts of colorants*, in textile processing alongside dyeing the 'wet-processing' includes also many other steps that might create environmental issues. Fleck (2020) highlights that just by replacing color compounds within the dyeing process is not enough. Also other compounds used in the process should be replaced with more sustainable ones and prosesses need to be developed further.

With textiles the colorant and fiber needs to bond to get a stable color. What about mordants? Are those used in the dyeing process with bacterial dyes? "Mordants are generally very very toxic or they are just purely metals" Fleck (2020) discloses. It is quite common that you walk into mordants if the color is not stable, but that effects on the sustainability of the dye. I asked Fleck if any mordants are used together with their bacterial dyes. "We focus on dye producing and we offer them to dye houses. These dye houses might use the dye with mordants" Fleck (2020) explains. Fleck (2020) says that they can't fully stop usage of mordants. If you're willing to stop the usage of mordants, good alternatives for that should be provided.

How new colorants are created with bacteria?

Constant research of new colors has been executed also in Vienna Textile Lab. Fleck (2020) tells that if they get a new color from the bacteria they need to figure out how the color behaves. Some of them work on textiles, some of them work better as food coloring or for other purposes. "That is just the nature of the chemical compounds" Fleck (2020) summarizes. In addition Fleck (2020) mentions that to be able to understand how well certain bacteria produced colorants behave on certain fabrics you need to understand the chemistry of each compound that the bacteria produces. You also need to be aware of some possible impurities that might come together with the extract even though you couldn't see them in the color itself.

Fleck (2020) describes that when they have a new color that they are able to produce with bacteria they will run tests with different types of fibers and that way they will define the baseline for the colorant. "When one compound is really wash-fast it is already a good sign" Fleck (2020) describes the testing. If the colorant doesn't stay on fiber well enough, they discard the bacteria. When the bacteria produced colorant has been tested, they bring it into the dyeing house and then the magic of the dyeing happens. At the dye-house they test various experiments with the colorant: changing of the pH-level, varying the temperature and maybe even some other trials. This is for understanding the chemistry behind the colorant.

"If everything would be solved already, where would be the fun of doing it, right?"

Karin Fleck (2020)

Figure 15. Bacterial dyed skarf by Vienna Textile Lab. Photo © Vienna Textile Lab.

Thoughts from the discussion

Bacteria colorants are certainly offering an intriguing alternative in the world of colorants and dyes. There are clearly beneficial properties in bacterial colorants which can raise them to a level other colorants can't achieve, especially when we think about environmental aspects. From our discussion I also understood that getting to the market with new colorant or dye innovations isn't that easy: it takes a lot of effort and money to get them through all the required standard testings. This is of course a tough situation for smaller companies and startups where the business is often based on innovations. It is clear that more work needs to be done so that we get more of these alternatives to the market. Luckily we already have companies like Vienna Textile Lab who are willing to take the effort and make a change.

Fleck (2020) also highlights the importance of multidisciplinary work. "Whatever education you have and you end up with this type of project, you're always lacking some sort of education. It doesn't matter what field you're from, because it's such a overlapping topic" Fleck (2020) explains. Fleck also mentions the importance of understanding different materials especially when it comes to ecological thinking with design. "Designers often match color to another that they want but what is behind it is left out--designers should understand what happens behind the color" (Fleck, 2020). This supports also my own thoughts of the importance of understanding the material especially when we work with new innovative materials. As a designer it is beneficial to understand the characteristics and procedures behind the material to be truly able to work with it. In addition to this, by understanding the background of the material, designers are also more proficient to tell about it for instance to customers and clients.

5 WHEN DESIGN MEETS SCIENCE

5.1 BIOFABRICATION AND BIODESIGN

In the design field a new term called biofabrication has been popping up in various contexts. According to Lee (2019) this term represents fabricating with biology and instead of using non-renewable resources to produce consumer materials we can grow materials directly with living organisms. It seems that this is also rising into public awareness. *"Designing with life"* as Lee (2019) names the whole movement is *indeed* a movement which incorporates design with microorganisms like bacteria, yeast, algae and fungi.

The notion of biodesign has been well encapsulated by William Myers in his book *Bio design: Nature, science, creativity* (2012):

> ***"Biodesign goes further than other biology-inspired approaches to design and fabrication. Unlike biomimicry, cradle to cradle (C2C), and the popular but frustratingly vague 'green design', biodesign refers specifically to the incorporation of living organisms as essential components, enhancing the function of the finished work."***

Emerging knowledge from life sciences is already offering adjunct potential for future fabrication and manufacturing. In addition to exploring the advantages of biological systems in terms of zero waste, reduction of energy usage and materials, we have great opportunities with synthetic biology, scientists have developed means to biofabricate like 'Nature' does. (Collet, 2016) With the help of synthetic biology we have new possibilities of programming living organisms and to grow new tailored materials. These new types of tools can offer new opportunities for design processes and as such lead us into a different future of materials.

For some people this might sound contradictory, for that reason it is important to remember that these matters evoke a need to re-evaluate the position and potential of design. Designers need to be aware of the ethical aspects of how to use these tools and what is the best way of utilizing them to build a more sustainable future.

Figure 16. Filtering colorant in a fume hood.

5.2 DESIGN IN THE FIELD OF BIOCHEMISTRY

Biodesign and biofabrication move in the field of biochemistry where design is bringing different types of viewpoint to the topics. Scaling up the focus from nanoscale to centimeters or even bigger in biochemistry is helping to see possible usages with the research, especially when the aim is to create something for commercialization. Design can offer a hand by turning the idea for instance into a concept or to a product. This can help with the marketing of the researched topic and by having a physical object or a practical concept it is easier to reach out for a wider audience. One could say that with this matter designers can work as a bridge and link source between fields and professionals.

To be able to work as a linking bridge designers need to have a good understanding of the researc topic. In this thesis work, my own aim is to learn and understand the field of biochemistry better. I want to understand better what is possible to be done and what are tools and protocols in this field. It's like collecting new types of tools into your own designer toolkit. With these tools I have more ways to approach design challenges and to have wider area of fields where to opetare. Once you get these tools to your toolkit and you know how to use them, you have a much better starting point as a designer to work again around these topics, like in this case within the sectors of biochemistry.

5.3 FINDING A COMMON LANGUAGE

As mentioned earlier, by collecting new types of "tools" a designer could have more capabilities to solve design challenges in the future. Before a designer is able to use these tools, she or he needs to know how to use them. Finding a common language between different fields is a necessity in this situation. According to Peralta (2017) in book Lost in Wood(s), finding a common language in interdisciplinary teams helps to overcome limitations of disciplinal jargon. Peralta also points out that interdisciplinary teams should always have a distinct common purpose.

From my own experience I have noticed that it takes some time to find out ways to express your ideas and thoughts in interdisciplinary team-working. This means that in different fields we are used to apply certain terminology that is not necessarily familiar for a person that doesn't have a background from that exact field or sector. In addition to this, people with different backgrounds might have different ways of working, practices that the other one might not have, and even a different native language. This of course requires a lot of new ways to express yourself in a way so that the other person understands your aimings and working habits.

To understand people from other disciplines, we might need to get familiar with one's working methods and procedures. From my viewpoint, the best way to understand these matters is by observing and by having open conversations. In my personal experience, finding a common language in the collaboration is a key for successful project. As Peralta (2017) highlights also, that it is crucial to clarify what are the objectives, goals and expectations

for each party taking part in the project. Already in the beginning it's wise to sit down and sort out these matters so that the project can move forward fluently. At first it might feel as a waste of time, especially when you're already excited to start the actual making. One key element in the collaboration is that everyone should feel appreciated and common trust between professionals is incomprehensibly important. However, it is equally important to be able to question matters that don't make sense to you while doing research. This should be reciprocal between the co-working partners.

This sort of multidisciplinary working is full of communicating. It can't be highlighted enough how important these conversations are especially when working in cross-disciplinary teams. (Groth et al., 2019). Constant reflection might feel tiring but it is an essential tool to keep track on how we are moving forward with research and development. At it's best multidisciplinary work can offer an advantage where complex matters can be tackled more easily, it nurtures creativity and the impact of the outcome touches a wider audience (Peralta, 2017).

Within this thesis work I'm going to focus on the collaboration between designers and professionals with a biochemistry background. With this work I'm willing to emphasize the power of working across disciplines and be open for challenges which might lead into new innovations.

In the following section I will bring out some comments from my mentors James Evans and Géza Szilvay who were guiding and helping me to build up this thesis project. Together with Evans we researched bacteria as a source for color and with Szilvay our focus was on researching colors of fungi. The answers from Evans and Szilvay (2020) concern our projects which were implemented in interdisciplinary teams. For me it was highly important to hear comments from my mentors how they experienced the project. These comments gave great insight from their side and also some useful tips for other design students and designers who are willing to implement a project like this. After the comments from Evans and Szilvay I will jump into making part where we work together to achieve colors with microorganisms.

JAMES EVANS
Laboratory technician
Aalto ARTS, Technical Services of the University

Have you worked with other design students/designers before? Which type of projects were they?
"Yes, they were almost all students from the Biomateriality course. There were 2 more involved projects, and they were both using fungus to produce scents. One of them for packaging materials and the other for perfumes."

What were the most exciting matters within this thesis collaboration / project to you?
"Switching to the high yield media and getting lots of dye/pigment was a very good sign at the beginning that this project could succeed. Working with small amounts of material can always be done, but it makes producing anything an impossibility. Also, the chemical purification went extremely well, when compared to purifying peptide dyes."

Were there any challenges with the communication as we proceed with the project? If yes, what were they?
"No, not really, but that is not the case with every project. The important thing in this project was that we were able to be realistic about what could be done, and to not get discouraged when results were bad. Very often, the first sign of serious problems causes others to abandon the project, when with a little more work or trying something new, it could succeed.
I sometimes say that getting used to failure is a success on its own. To collaborators who do not have this experience, they often read something and think that it should be easy and fast. It is one thing to read, and a very different thing to do. It was also very important that you were flexible in your time and expectations. Especially since we ended up having to close the project a month early. The fact that it still managed to succeed means that work went well!"

Did this project offer you something new or exciting?
"Yes, it did. We got to try out the preparative HPLC (granted, it did not work). But still, it was important, because getting it to work means that many other, different bioart projects can benefit. It could, for example, be an excellent solution to the problem of purifying peptide dyes. Plus, I just like doing this kind of work."

What are your wishes/notices for designers who are willing to utilize biodesign/biofabrication in their design work and willing to collaborate with biology scientists/biochemists?
"Be realistic with expectations, be willing to dedicate a lot of time to the project. I had mentioned this before in a different meeting, when the question was 'what kind of student succeeds in your lab', and my answer was 'the one that shows up'.
I (and others like me) have lots of competing interests and projects. I can't take someone's work more seriously than they do, and if they can't take it seriously enough to make time for it, then I won't do it alone. Professional designers might have the resources to fund a lab, but students have only their time."

Do you have any additional comments regarding the project?
"It was a lot of fun, and very successful. I think what you did here is a very good starting point for further work, either here or elsewhere. It is rare that a Master's project gets so far along in such a short amount of time."

GÉZA SZILVAY
Senior Scientist
VTT, Industrial Biotechnology and Food Solutions

Have you worked with other design students/designers before? Which type of projects were they?
"I worked together with designers in a project where we made a demo product from microbially produced materials. The designers were from a design company and it was interesting to see how their interaction with materials differed from ours. To make the demo product the designers had to come to the lab and understand how the new materials were produced. They were interested in the haptic properties and how certain forms can be achieved with each materials. We researchers of course are more interested in the quantitative material properties, such as strength or surface properties. For us researchers it was very useful to learn what things are important in practice when developing new materials so that they can be applied for example in consumer products.

Earlier I have been working with a design student from Aalto University. In this collaboration the designer worked closely with the microbial organisms that created the materials. The student made the effort to learn how to cultivate the organisms and how to process the materials. These collaborations taught many people in our organization that the involvement of designers in material research can be very fruitful."

What were the most exciting matters within this thesis collaboration/project to you?
"It was very interesting to note that the molecules we produced already had a visual appearance and how this then lead us to rate their appearance as pleasant or unpleasant. Typically the molecules we produce are just chemistry without any feelings attached to them. It was also very interesting to link so tightly the end application requirements with the choice of microbial species."

Were there any challenges with the communication as we proceed with the project? If yes, what were they?
"Obviously the terminology and language are challenges as in any interdisciplinary work. It was very beneficial that the student had previous lab work experience, also on working with microbes. In general, I felt that the scope of the project was sort of being defined and re-defined during the project which is a very different approach from our typical projects where the scope and goals are quite fixed from the beginning. These changes were, however, well communicated by the student."

What are your wishes/notices for designers who are willing to utilize biodesign/biofabrication in their design work and willing to collaborate with biology scientists/biochemists?
"Designers aiming to work with biodesign and biofabrication surely benefit from the expertise and access to equipment provided by scientists. I believe, most scientist would be open for collaborations. There's however a challenge in communicating the goals of designers to researchers as many of us still might think design simply as product design or graphic design.
We needed a concrete collaboration to start to understand what designers can bring to the table in materials research beyond demo products. In my experience it is important that the designers would be ready to spend time in the lab trying to understand the materials or organisms at a basis level, perhaps even at the gene sequence level. Unfortunately, it is still challenging to establish collaboration for long term, partly because funding does not encourage this collaboration. But also there is still work needed to be done to bring these two disciplines closer together."

6 CREATING COLORS WITH MICROORGANISMS

6.1 OVERVIEW OF THE PROJECT

This colorant making part of the thesis is explained through the project which was done with bacteria in Biofilia laboratory. In the middle in section *6.4 Case: Working at VTT* I'll introduce a case study that was implemented in VTT Technical Research Centre of Finland Ltd facilities by utilizing fungi as a source of color.

Within this chapter I will be describing in detail how I implemented a color palette together with living microorganisms and with an invaluable input of biochemists. I will go through each step of the process and share with visual photos and figures what the color results are from each procedure. In-between the text, I'll place parts of my daily journal that describe my own thoughts and findings throughout the project.

Figure 18 sums up the working process and steps while working with microorganisms in this project. These steps were followed while working with both bacteria and fungi. Steps (A), (B) and (C) are opened up in chapter *6.3 Making of Colors* and steps (D) and (E) are described in chapter *6.5 Color Experimentation* and in chapter *6.6 Color and Material*.

Figure 17. Photo of placing fungi from a plate into a nutrient broth.

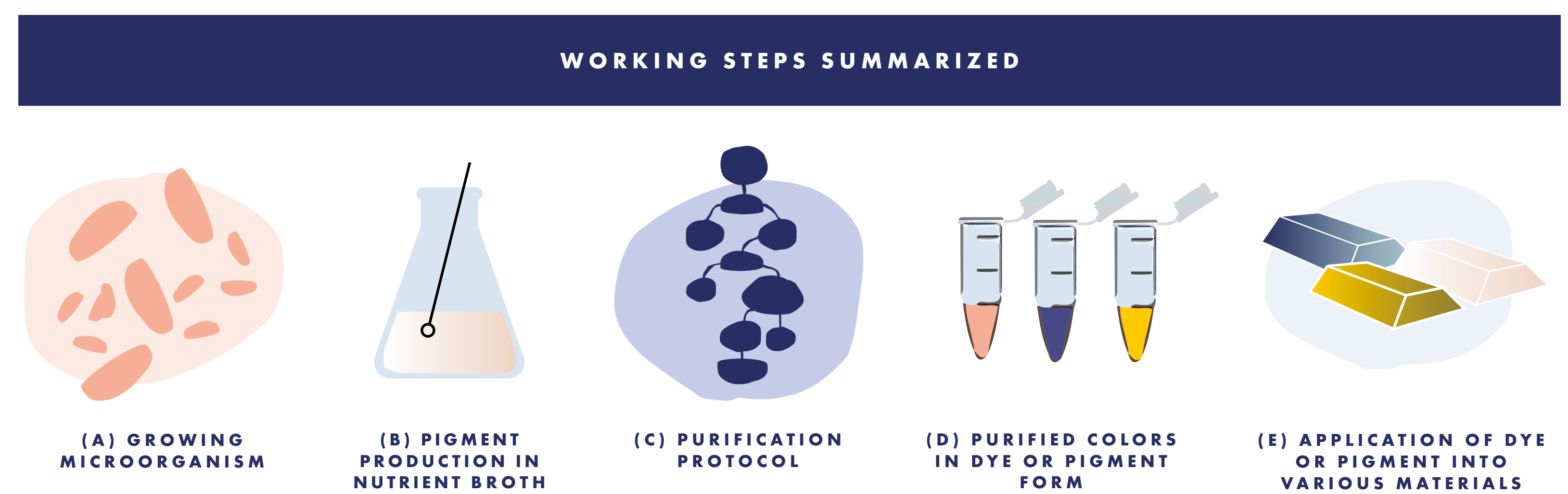


Figure 18. Chart of the working process and steps while working with microorganisms. © Eveliina Juuri.

DIARY NOTES FROM WEEKS 45-48

8.–29.11.2019 Biofilia ABC course

Even though I do have some experience of working in a laboratory environment, the nature of laboratory work in Biofilia lab compared for instance to CHEMARTS laboratory is totally different. Working in a biochemistry laboratory is much more strict and procedures are so much subtitled. While working in the lab, it's always good to make sure that you know what you are doing and also let others know what you're doing. From a designer's perspective, it's sometimes hard to remember that you can't just make your craziest experiments within the materials and in the laboratory environment but with some time the procedures become more natural and working in the lab is easier.

As the first part of the Biofilia ABC course we read safety instructions for laboratory working and took an online test to confirm our learnings. After the online part we were able to come to Biofilia lab for the next part which was about laboratory working. All of the exercises were certainly useful for my thesis project and by doing different types of trials during the intro course, I also got more confidence to work in the laboratory environment.

When it comes to working with the scientists I think that as a designer I don't have to understand every detail that happens during the making. Although I do need to know where we are aiming for and keep in mind the overall picture of the whole project. When you're working as a designer in a multidisciplinary team, it's crucial to also understand other fields. It was a good decision to educate a little bit of myself in laboratory work so that I have a better understanding of the terms, working methods, timings, complexities and challenges.

6.2 BASICS OF LABORATORY WORKING IN BIOCHEMISTRY

To be able to work in a laboratory environment you need to know the basic rules before entering the lab. Before starting my thesis work I decided to take a laboratory intro course for Biofilia Lab that is a base for Biological Arts. It's a biological art unit that was launched in the School of Arts, Design and Architecture in 2012. This Biofilia ABC course was held by the laboratory manager, James Evans. After the introduction course I had access to work in the laboratory under supervision.

When working in a biochemistry lab the safety guidelines and general efficiency in the laboratory work need be taken into consideration (Fig. 19). After the basic introduction I had a better understanding of the working methods and which guidelines I should be following to be able to work safely while operating in the laboratory (Fig. 20).

Figure 19. Photo from the Biofilia ABC course.
Figure 20: Biofilia laboratory. Photo © Aalto University.

6.3 MAKING OF COLORS

Setting up a project plan

After taking the Biofilia ABC course I was able to start my work with the help of the lab manager, James Evans. I consider this way of working more as a collaboration, since Evans was putting a huge input also to this project and helping me throughout the project.

We started the project by making a clear plan of all of the steps we're about to have. I needed to be able to spend quite many hours in the laboratory and be also flexible with my schedule in case things didn't work as expected. It was helpful to plan the schedule for the project together with Evans since I didn't have the experience of working with living microorganisms before. We narrowed down what are the goals, both of our schedules and what could be the possible experimentations if we have extra time.

Because we are working with living organisms that have their own will, we can't really be sure how the project is going to progress. We of course have some practise based knowledge (Evans from biochemistry and me from design experimenting) how long each working step would require time. In addition to these we had scientific articles and recipes to use while implementing the research itself. Nevertheless, often something unexpected might happen when you're doing something experimental but that is part of the process and we learn as we move forward.

Figure 21 shows what was the expected timeframe with each bacteria that we were planning to work with. This timeframe was for estimating how much time we should be reserving for creating one color with the bacteria. This also includes experimenting with materials to be able to tell more about the characteristics of the colorant that was built by the bacteria. This way we could also get more valuable information for the following procedures.

STEP	DAYS	CONTENT AND EXTENT OF THE WORKING STEP			
LIQUID MEDIA	2-7	PREPARING	AUTOCLAVING	INOCULATING	GROWING / EXPERIMENTING
CELLS	1-3	EXTRACTING	PURIFYING		
DYE / PIGMENT	2-X	CHARACTERIZATION	EXPERIMENTING WITH MATERIALS		

Figure 21. Schedule estimation with one bacteria.
Figure based on a schedule estimation by James Evans (2020).
Figure visualization © Eveliina Juuri.

DIARY NOTES FROM WEEKS 49-52

2.12.2019 Meeting with Noora Yau
Today we met with Noora Yau who provided me helpful information about her own experience of working together with her multidisciplinary team in the fields of chemical engineering and design. Currently Yau is just starting her PhD work together with her team and it was extremely remarkable to hear also her experience of the ongoing topic that she's working on. Yau also provided me some tips and useful readings that I could utilize in my project.

I feel already at this point that it's crucial to hear others thoughts of the project and to hear some feedback on the topic framing. It's also nice to natter about the communication flow and how the project is progressing.

20.12.2019 Just a Thought
Designer needs to lead the way: who else would make a move? Who else would show the idea? Who else would prove it? We need to work as a bridge. Link fields together and try to find a common language. This is a key for successful collaboration and platform for new innovations. If we only work as individuals without any collaboration, I claim that nothing too revolutionary is not going to come up. Why would you try to find something totally new and ground shaking on your own when you can figure it out together?

Background research of the bacteria

For this project we chose bacteria species based on their color that they're able to produce. Luckily Evans already had some bacteria stored in the lab that we could start working with. In the beginning we chose to work with:

***Serratia marcescens* – Prodigiosin**
***Pseudomonas fluorescens* – Pyoverdine**
***Janthinobacterium lividum* – Violacein**

Each of these bacterias are able to produce a certain pigment. These pigments, color molecules, were identified in earlier studies so we could expect what color molecules we were about to get from each bacteria. In the beginning of the project I marked myself what my own expectations/assumptions for the colors from each bacteria were (Fig. 22). I also listed some properties that I would like the color to have (Fig. 23).

All of these bacterias are well researched so we were able to find multiple research articles of each bacteria and especially articles that consider pigment production. Scientific articles and reading of them was quite a new world to me to be honest but it was definitely worth learning – through this I found a whole new world with interesting findings and ideas that are helping people to do their research all around the world. This gave me tons of new ideas and it was almost overwhelming how much I was about to learn within this thesis topic.

Evans encouraged me to find scientific articles that would show the purification protocol for the pigment isolation. At first it felt difficult since the language in the articles seemed cryptic to me–to say the least–but I tried to look for things that showed detailed information for the protocol and maybe even colored pictures of the color results.

Beginning of the color making

After the background research we were able to start the project. We re-streaked all the old bacteria cultures onto new agar plates and placed them to grow at room temperature. We were growing all the bacteria species simultaneously but since the growing varies we selected *Serratia marcescens* as our first bacteria that we started to grow in bigger quantities (Fig. 24).

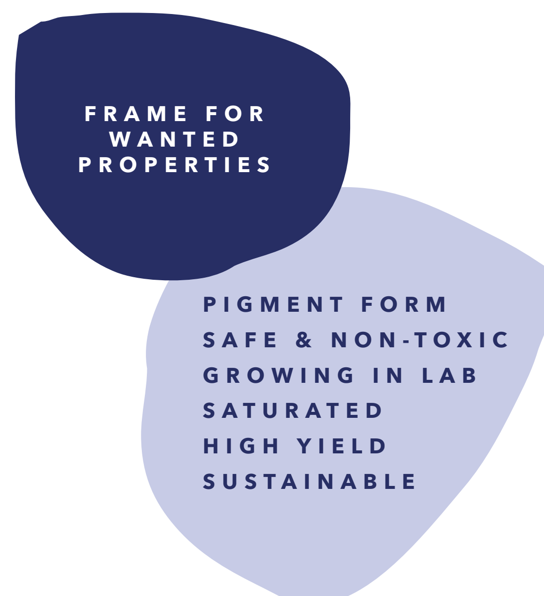


Figure 22: My color expectations from each bacteria.
Figure 23: Frame for wanted properties for the colorants.

Figure 24: Photo of agar plate with S.marcescens and structural formula of prodigiosin.

DIARY NOTES FROM WEEKS 1-4

10.1.2020 at Biofilia lab

I took my camera with me to the lab so that I'd be able to document the whole process while we work. We also re-plated all the bacterias into new growing plates that contain the "food" for the bacteria in them. Re-plating happens with a loop and we just pick up one colony of colored bacteria and spread it on top of the agar surface. James had these bacterias already growing in the lab, so we just needed to take care of these cultures and make sure that they are doing well. This way we can also make sure that we have enough of them growing in case something goes wrong with the experiments.

13.1.2020 at Biofilia lab

Today we tried out to extract color out from the growth medium. We had an expectation that the bacteria pigment should have a red-orange color, but in the growing medium it appeared as pale brownish-orange color (Fig. 25:A). After we centrifuged the growth medium liquid with the bacteria in it we could see some reddish color in the pellets but the amount of color was relatively small (Fig. 25:B). We saved the pellets but discarded the supernatant that didn't have any color in it.

When we had only the pellets with the hint of color we needed to check that the pH-level is correct. After separation (Fig. 25:C-E) we had a pale pink color in purified liquid and after concentration this liquid we were left with salmon pink oil (Fig. 25:F). The amount of the oily liquid was so little that it increased a question if we should try something else to be able to achieve better results color wise.

14.–15.1.2020 at Biofilia lab

James walked into me while I was going to a meeting. Since he walked out from the supermarket, I thought that he was buying some food for himself but the next day he told me that he did some shopping for the bacteria experiment. Turned out that James had found an article that was about pigment production with S. marcescens bacteria and he went to buy some ingredients for the experiment. Seemed that these new incidents were making the bacteria produce bright pink pigments (for unknown reasons) but of course I got very excited about this, especially when the previous batch didn't produce as much color.

17.1.2020 at Biofilia lab

Today we got amazing results from the S. Marcescens bacteria! We extracted the pigment out from these bright pink flasks and the pigment turned out to be super bright and vibrant. This was a huge success! We purified the liquid and in the end of the day we got some powdery pigment out from the bacteria and I was even able to paint with it, like with water colors.

Looking for the right growth medium

Figure 25: First extraction experiment with S.marcescens.

Creating colors with bacteria

As mentioned in the diary notes, Evans found a new recipe that was using a different type of growth medium for *S.marcescens*. This recipe was written by Giri et al. (2004) *A novel medium for the enhanced cell growth and production of prodigiosin from Serratia marcescens isolated from soil*. By utilizing the growth medium from this recipe we got excellent color results already from the very first batch.

In the following sections I'll introduce visually the colorant making protocol that we were following to extract prodigiosin pigment out from *S. marcescens*.

A. GROWING BACTERIA

Different microbial species can be ordered from banks that have a collection of different microbial species. Within this case, we already had *S. marcescens* in the Biofilia laboratory and we knew that it's safe to work with. We started to grow bacteria in LB (Luria-Bertani Broth) agar plates. This provides a nutrient growing surface for the bacteria.

It's worth mentioning that not every bacteria produces this bright color but for this project we have selected ones that do produce color pigments. This makes working also easy because we're visually able to see how well the bacteria is growing (Fig. 26). As soon as we see bacteria producing colored pigment we are able to move into nutrient broth.

Figure 26: Serratia marcescens on a LB agar plate.

B. PIGMENT PRODUCTION IN NUTRIENT BROTH

After the bacteria has grown nicely we were able to streak a piece of it into a bigger batch where it can multiple itself more freely. This batch is for growing the bacteria in a bigger scale where pigment gets produced in bigger quantities. As mentioned before, our first experiment didn't provide us desired results but by changing the growing medium into a different one the bacteria was able to produce a great amount of pigment in the nutrient broth.

It was clear that with this growing medium we were able to get a much higher yield of color pigment. For this project it was a big turning point that Evans found and tried out this recipe since after that we were able to get bright pink color in bigger quantities (Fig. 27). When the bacteria has grown enough we could already use this liquid as a colorant after remowing pellets (Fig. 29:M) but since the aim was to have a purified pigment, we move into purification.

Figure 27: Serratia marcescens in nutrient broth.

C. PIGMENT PURIFICATION PROTOCOL

Purification protocol is for isolating the color pigment out from the bacteria. In other words, we separate the color out from the bacteria. As yet the bacteria has been alive but during this procedure only the color pigments are going to survive. This is because anything living can't be taken out from the laboratory and we also want to be sure what's in our colorant. For the purification protocol Evans created a chart that we followed while isolating color pigment prodigiosin from the bacteria. Since this part consists of multiple steps and the working methods inside this part are relatively unknown for most of the people in the design field, I'll visually narrate the steps for the purification protocol on next spread (Fig. 29).

After the purification we had our final product which is a bright pink prodigiosin in a pigment form that is possible to apply into various materials or use it as it is for instance as a paint by soaking it into some solvent. I also want to highlight that in this stage no bacteria is left in this pigment.

Figure 28: Solvent for purification.

Purification Protocol

For Serratia marcescens

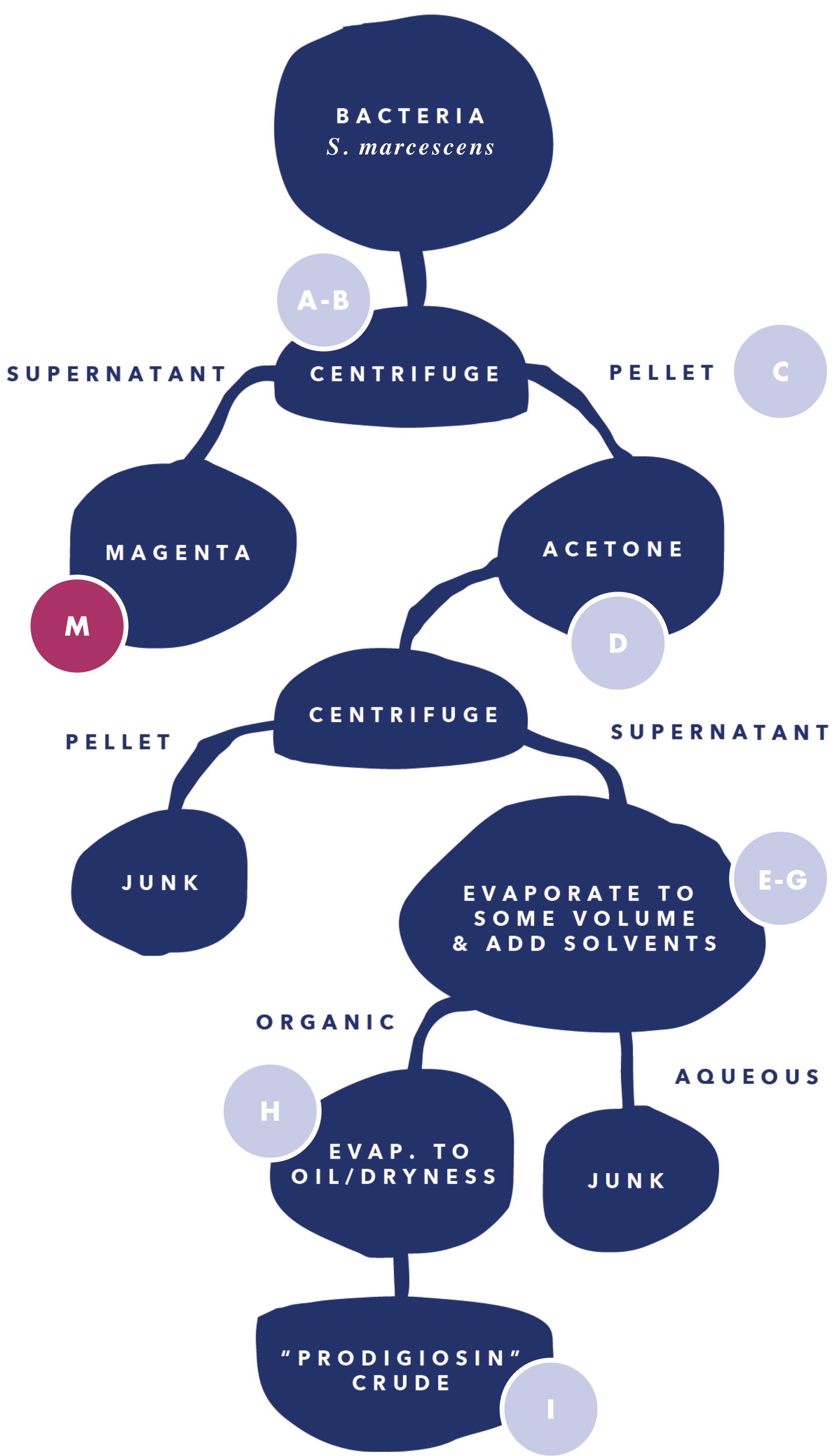


Figure 29. Adaptation of the "Purification protocol for S. marcescens figure" by Evans (2020). Adapted with permission. Visualization © Eveliina Juuri.

6.4 CASE STUDY: WORKING AT VTT

In the beginning of this thesis work I heard about an opportunity to take part in an ongoing project that was researching bio-based colorants and natural sources to achieve color. VTT Technical Research Centre of Finland Ltd was taking part in this project and I had a chance to join the research team. Since I had an interest towards working with microorganisms this was a great match because VTT was planning to implement a research with fungi, which as well can work as a source for color.

This working opportunity offered a nice insight to the scientific research world where I was mentored by Géza Szilvay, Senior Scientist at VTT with a focus on researching biotechnological solutions for new biobased materials. All the color results will be part of the ongoing *BioColour – Bio-based Dyes and Pigments for Colour Palette* project that is founded by The Strategic Research Council (SRC). This research will continue until year 2025.

As an overview of the process the focus was to find natural colorants together with fungi (Fig. 30). As bacteria, also fungi is a microorganism that is able to produce color pigments while it's growing. VTT already had previous experience of working with fungi as a material and based on that knowledge we had a good starting point for searching colors from species that were already in VTT's stock.

Throughout our working I also collected all my notes into a laboratory notebook. This is a practise that is commonly used in scientific research. Lab notebook was a helpful tool to keep track of the project, especially when I was working simultaneously in two different laboratories (Biofilia and VTT's laboratory) with two different projects. This also made repeating of the experiments possible, for instance if we wanted to produce more colorant with fungi.

Since I have introduced the process of working with microorganisms earlier in this paper, I won't go too deep into the working procedures. In the following sections I will briefly introduce how we searched for color by utilizing fungi as a color producer and what were results so far. I also want to point out that some of these results are confidential and that is why I'm not able to share all of them since the project will be further continued by VTT.

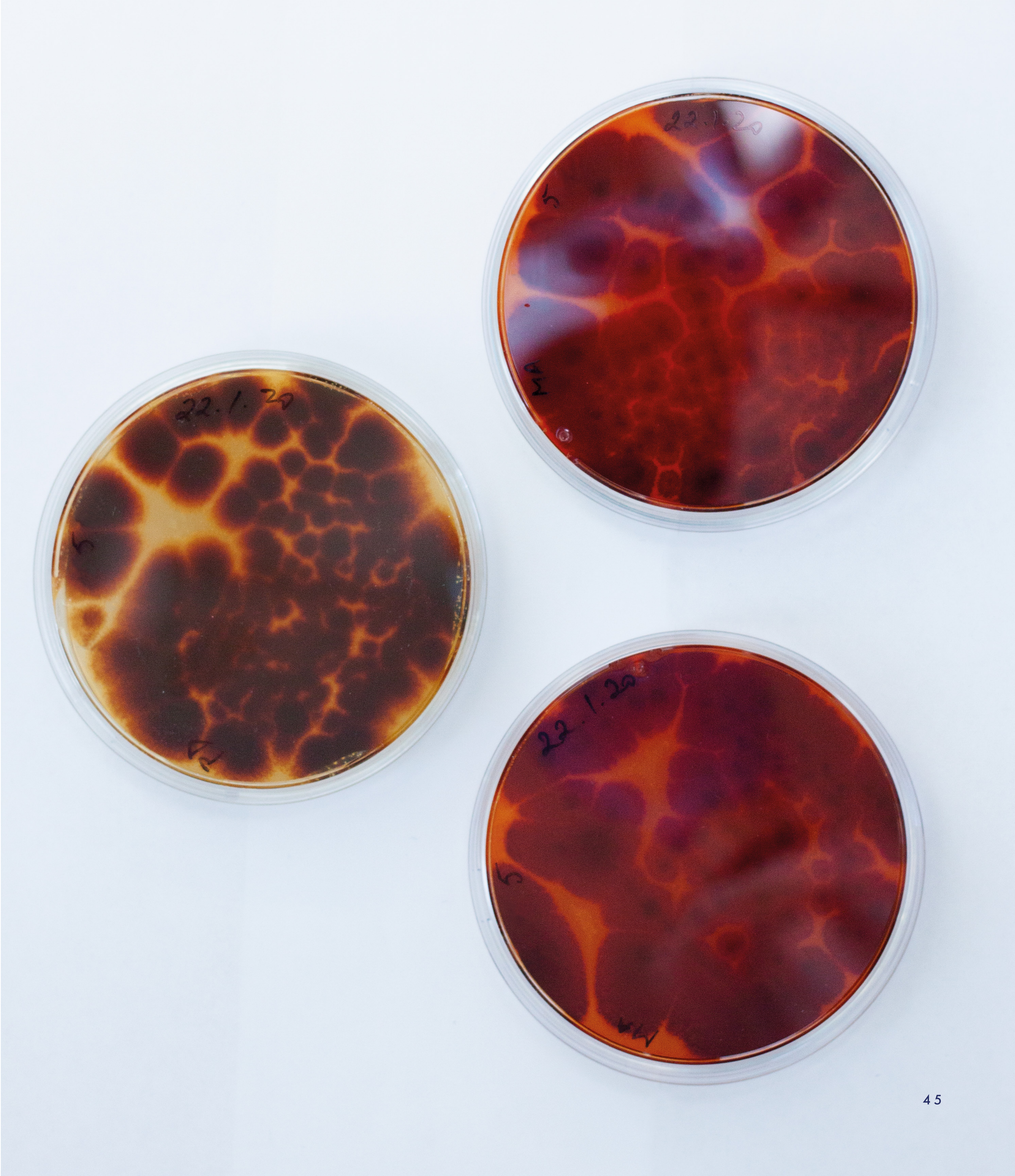


Figure 30. Fungi can grow differently on different growth mediums.

Figure 31. Filtered and freeze-dried colorant from fungi ready for soaking.

Figure 32. Soaking test with fungi colorant.

Working towards color

In the beginning of the project we selected species that were noted to have a certain color while growing in a laboratory environment. These selected species were streaked onto agar plates and after the fungi was well grown, we were able to collect a small piece out from the culture and place it into a nutrient broth. We were using a selection of different types of nutrient mediums to be able to tell which one works best with each fungus for color production.

After a few days of growing in an orbital shaker, we were able to tell if the fungi was producing any color. Since I had only a little experience of working with fungi as a source of material, I didn't quite know what to expect from the colors in the beginning of the project. However quite soon I noticed that with fungal species, which we were utilizing for color production, colors were quite close to plant based colorants: tones were warm, settled and earthy. Once the mycelium was well grown and we had some visual color in it, we were able to start the color isolation. For this step we utilized different methods, where soaking was one part of the procedure (Fig. 33). We also evaluated together with Szilvay whether colors were appealing or not.

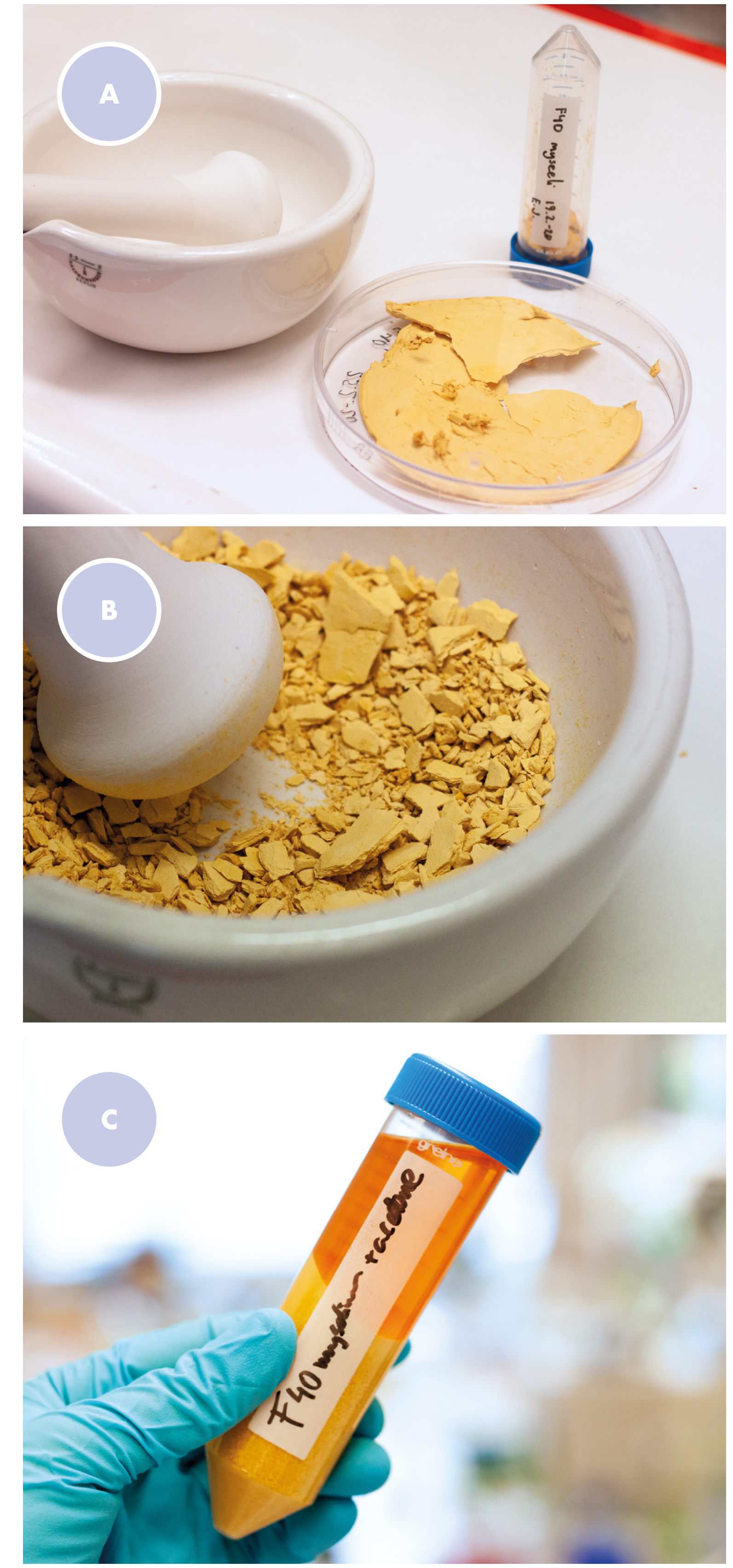
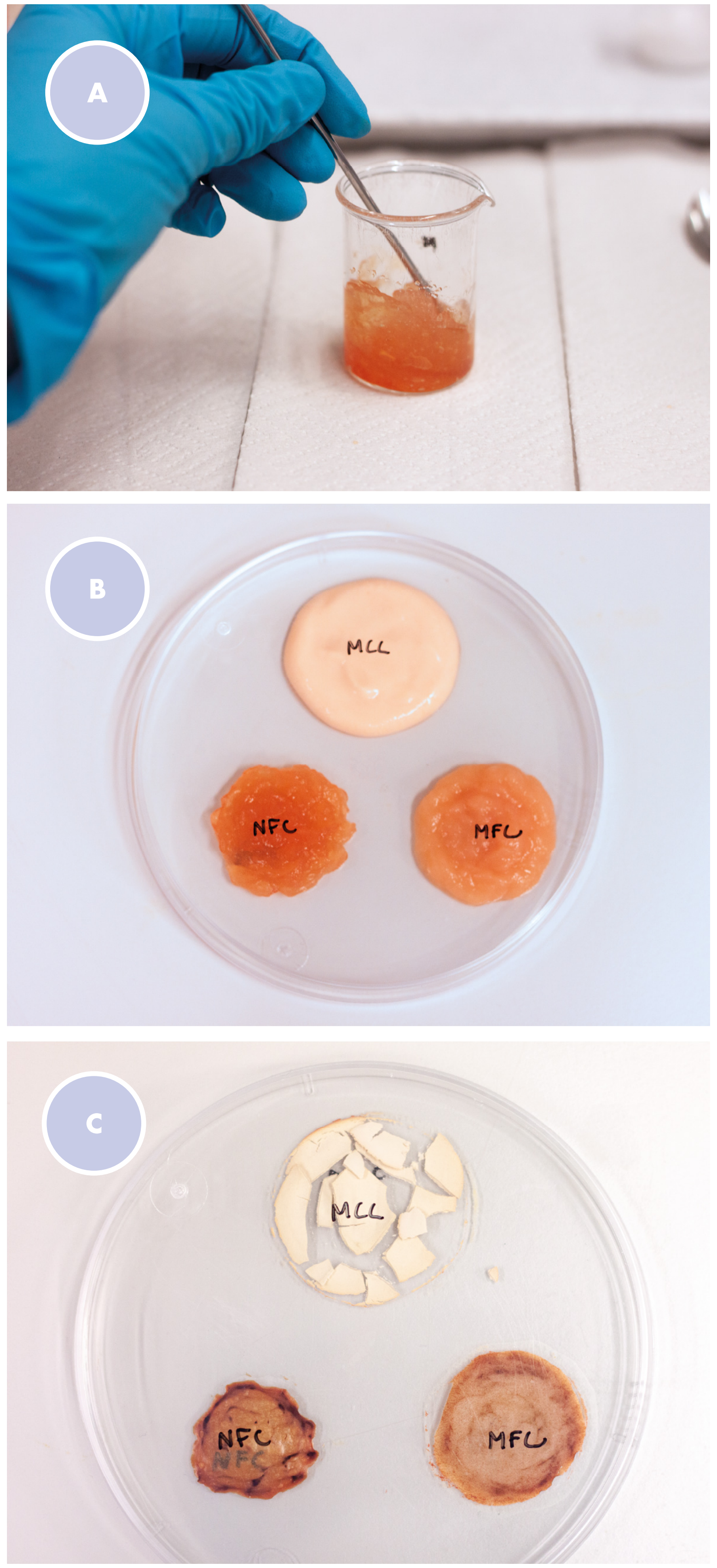


Figure 33:A-C. Soaking colorant from the mycelium into acetone.



Preliminary material experimenting with the colorants

I was able to implement some preliminary tests with the achieved colorants together with yarns and textiles without any mordants. I also implemented a small experiment with the colorants in different cellulose materials: NFC (nanofibrillated cellulose), MFC (microfibrillated cellulose) and MCC (microcrystalline cellulose) without any additives. We also protected all of the samples from direct sunlight since it might have an effect on the color results.

As we can see in the photos on left (Fig. 34:B), the purified fungi colorant colored different cellulose masses well with a relatively small amount of colorant. However when the masses dried the color turned out patchy and the tone was shifting to a more brownish color. This may have been caused by the mixture of cellulose based materials together with the colorant or the colorant has this type of character when it dries, or both.

This was a good example of a material experiment that already told something about the characteristics of the colorant that we couldn't see from the colorant while it's in a liquid form. Since colorants are often applied into something with various methods, it makes sense to experiment also with diverse materials to be able to tell how well colorant performs without any additives.

Figure 34:A-C. Fungi colorants in cellulose based materials.

Colorants from fungi

Within this timeframe we were able to find colors that showed potential for further investigation where the properties of the colorants should be researched more as the project continues. Colors with fungi produced colorants were othen having a warm undertone from yellow to red and brown (Fig. 35). We also found some light pink color but the paleness of this color narrows down possible application purposes since the color wasn't highly pigmented.

At this stage of the research, we are not yet able to share the exact numbers of how concentrated these colorants are and if they include more than just one color molecule, but we do know the procedure for making these colorants and of course visually we were able to tell how pigmented or saturated the color was.

Because of this short time period that I was able to take part in this project, I wasn't maybe fully capable of seeing the full potential of these colorants but I'm happy that we achieved some beautiful colors with fungi. It is clear that these colors are offering a good option for natural colorants. As the research progresses I hope that more of the characteristics and properties of these colorants will be investigated to be able to describe their full potential. I also look forward to seeing what is the range of colors that can be achieved together with fungi.

On the next spread I'll present the colorants that were created within this project (Fig. 36).

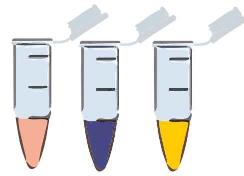
Figure 35. Fungal colorant extracts in solvents.

FUNGI COLORANTS
Diluted in solvents

1. Colorant A in 60% EtOH (ethanol)
2. Colorant A2 in 60% EtOH, 1/10 dution from colorant A
3. Colorant F39 in acetone
4. Colorant F40 in acetone
5. Colorant F43 in acetone
6. Colorant F43 in acetone (color varies)

Figure 36. Different fungi colorants in solvents.

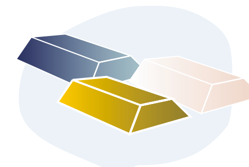
6.5 COLOR EXPERIMENTATIONS



We are able to make measurements for identifying the color pigment *prodigiosin*. From the chemistry side we can find instruments for diagnosing the exact color molecule. For this purpose we used spectrophotometry devices and high-performance liquid chromatography (HPLC) technique (Fig. 37). Spectrophotometry device is for measuring how much a chemical substance absorbs light (Chemistry Libre Texts, 2020). High-performance liquid chromatography technique is for separating, identifying and quantifying components that are in a mixture (Chemistry Views, 2020). By identifying the pigment with these techniques we got data which confirmed that we are working with prodigiosin.

We also implemented a pH-test with the pigment that showed us how the color changes in acetone (pH 7) from acidic (<7) to basic (>7). In acidic acetone solution the color turned more purple whereas in basic acetone solution color it yellow (Fig. 38).

6.6 COLOR AND MATERIAL



I think color and materials always walk hand in hand and each have an effect on another. That is why I wanted to apply the colorants into materials to be able to see how they affect one of another. It's also crucial to experiment with the colors to be able to tell more about the characteristics of each color. Since we have colorant as our material, it is essential for the design process to explore and experiment already in the early phase to be able to understand the material properties, performance and behavior (Härkäsalmi, 2017). By applying the color into different materials it makes the color also more tactile and we are able to tell more about its properties.

For my color application materials I chose materials that are offering a wide range of properties and materials that can be considered as biodegradable materials. Within the next chapter I'll introduce photos of my material experimentations that were colored with various methods by using prodigiosin pigment as a colorant.

Figure 37. HPLC column and HPLC machine.

DIARY NOTES FROM WEEKS 5-8

29.1.2020 at Biofilia lab

It was time to characterize the pigment molecule. We had reserved a chromatography from the Chemistry department where Leena Pitkänen was introducing us to the machine how the program is working. With this machine we would be able to characterize the exact color molecule (expected to be prodigiosin) that we are working with but unfortunately we had some difficulties to get the program running and that's why we needed to delay the characterization for later time.

6.2.2020 at Chemarts lab

Today we went to CHEMARTS lab to do some trials together with our final pigment! I wanted to mix the color with different types of biodegradable masses to see how it affects the color and how well it behaves as a colorant. We left all of the material experiments to dry in the lab into a closed cabinet so that the sunlight wouldn't affect the color.

12.2.2020 at Biofilia lab

We made a pH-test together with the pigment. We prepared small test tubes together with the pigment in acetone. We added potassium hydroxide (KOH) in methanol (MeOH) solvent into acetone that we then added into the test tubes. We increased the pH slowly within each tube by adding small amounts of the KOH+MeOH+C3H6O solvent. In the end we had a nice collection of tubes with different pH and we could clearly see how it affects the color. This was a great trial to make since the pH-level affects the color. This test already shows how to stabilize the color is.

Figure 38. PH-test together with the pigment in acetone solvent.

7 BACTERIAL COLORANT RESULTS

7.1 MATERIAL EXPERIMENTATIONS WITH PRODIGIOSIN

Figure 39. Washing test with the colored textile.

DIFFERENT TYPES OF TEXTILES
With prodigiosin pigment

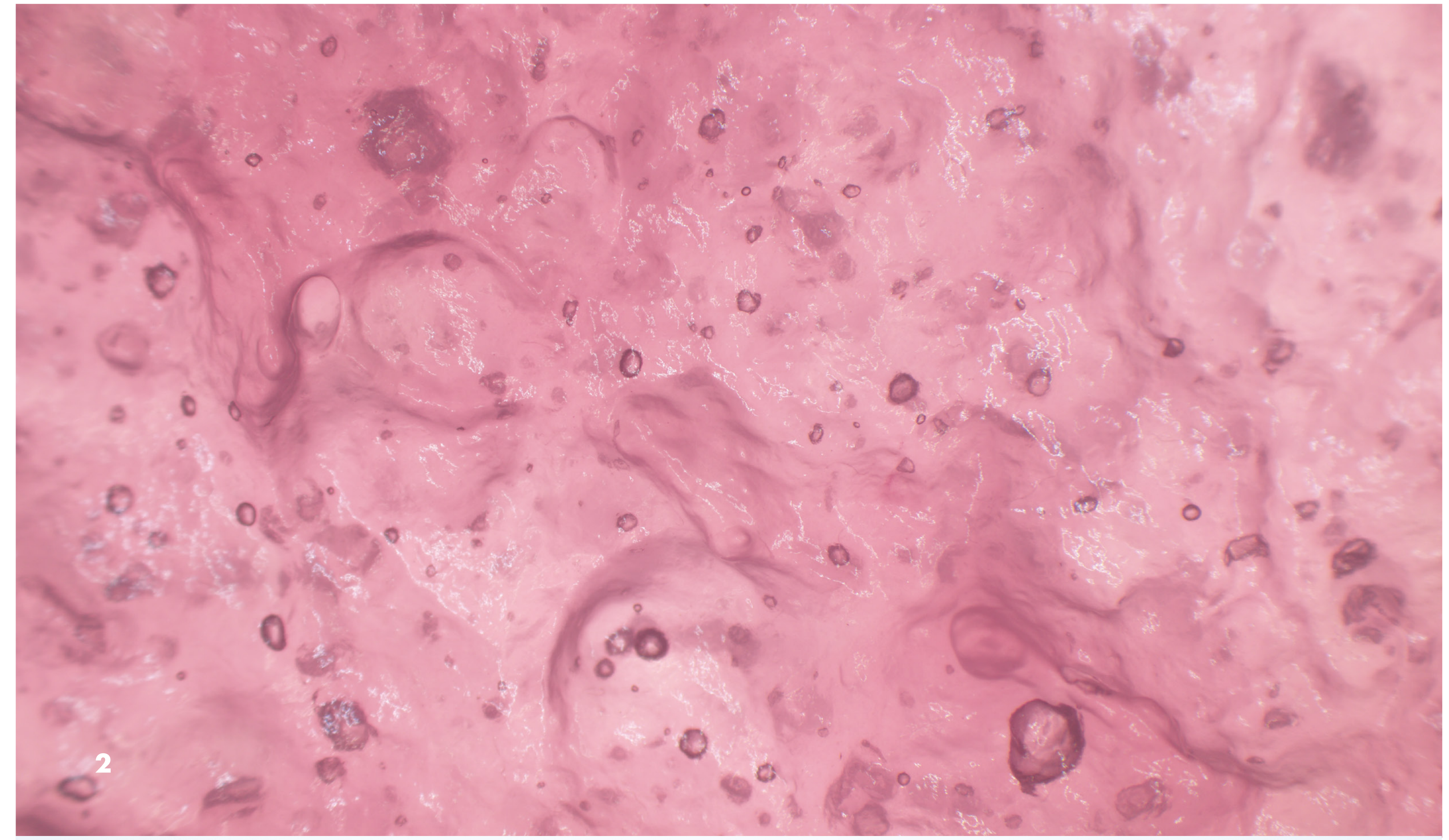
1. *Velvet Cotton (100% CO), printed pigment*
2. *Worsted Twill Wool (100% WO), dyed fabric*
3. *Velvet Cotton (100% CO), dyed fabric*
4. *PLA fabcir, dyed fabric*
5. *Worsted Twill Wool (100% WO) + PLA fibers, dyed fabric*
6. *Satin Silk (100% SI), dyed fabric, hanged to dry*
7. *Satin Silk (100% SI), dyed fabric, dried on surface*

DIFFERENT TYPES OF COATINGS ON PLYWOOD
With prodigiosin pigment

1. *One layer of pigment*
2. *Two layers of pigment*
3. *PLA & pigment*
4. *Oil & pigment*
5. *Beeswax & pigment*

MICRO- & NANOFIBRILLED CELLULOSE
With prodigiosin pigment

1. MFC, colored with pigment
1. Microscopic photo of wet NFC and dryed sample, colored with pigment
2. NFC, colored with pigment
3. NFC & high concentration of pigment



BIOPLASTIC: PLA
With prodigiosin pigment

1. Melted PLA fibers, colored in acetone & heated with the pigment
2. PLA fibers, colored in acetone with the pigment

1

2

CELLULOSE PULP
With prodigiosin pigment

1. Microscopic photo of colored cellulose pulp with the pigment
2. Dryed cellulose pulp with the pigment

Note: Bleached cellulose pulp

1

2


S. Marcescens
E. JUVRI
RED II


S.marcescens
RED II
E.JUUR.I

DIARY NOTES FROM WEEKS 9-12

4.3.2020 Just A Thought

This whole project is a travel out of your comfort zone – it's a new field, new material, new people and new working methods. I have never felt this insecure but I think that's highly valuable experience. It's not only something that I can treasure in my memories but it's actually something that gave tons of new tools to my design toolkit that I can use in future. Sometimes it's demanded that you come out from your comfort zone and face some of the hardest situations with a right attitude to be able to achieve something thrilling.

I would say that all the learnings from these projects were the most valuable thing that I can get out of them. It's the key for everything: in the future I know how to look for help, in the future I know better what are the protocols and how I should prepare myself for this type of work.

13.3.2020 at Biofilia lab

Situation in the world is running into a more serious state: coronavirus, also known as Covid-19 is now spreading dramatically and it seems that soon we are in the stage of pandemia. This of course means that schools are shutting down and also mine work in the school lab needs to end at least for now.

16.3.2020 at Biofilia lab

Today we needed to close down the Biofilia lab and freeze the bacteria that we have been using for my thesis project. Basically this means that I won't be able to work in any laboratory anymore and my experimenting with the bacterias and fungi is done. Luckily we still had some prodigiosin pigment left so I can keep on experimenting with that at home.

Saddest but also gratifying thing is that we just got promising color results of Janthinobacterium lividum that we have been growing this whole time without getting any color (Fig. 40:A). Luckily we got it growing but sadly we needed to also freeze this one for later use. (Fig. 40:C).

7.2 EXPECTATIONS VS. ACHIEVEMENTS

Because of the nature of working with living materials we were not able to produce some of the color that we were looking for. In the beginning of the project we started to grow *J. lividum* to get violet color from it, but for unknown reasons we didn't get it to produce color. By ordering a new one we were finally able to see bright violet color but at the same time we needed to wrap up the project. Also usage experiments with *P. fluorescens* bacteria needed to stay for next time.

Nevertheless, the project with bacteria provided me with the brightest color that I couldn't have expected. Prodigiosin pigment and different fungi colorants showed colors that surprised me positively even though I knew that these colors are possible to create with microorganisms. It was learningful to see how colors were created with microorganisms and what is the needed effort for this type of work.

Although we did have some challenges while working with microorganisms I think it was part of the progress. When we try to understand a new material we constantly learn something new and difficulties and failings are part of it. All in all, these projects exceeded my expectations and most importantly it gave me joy through success.

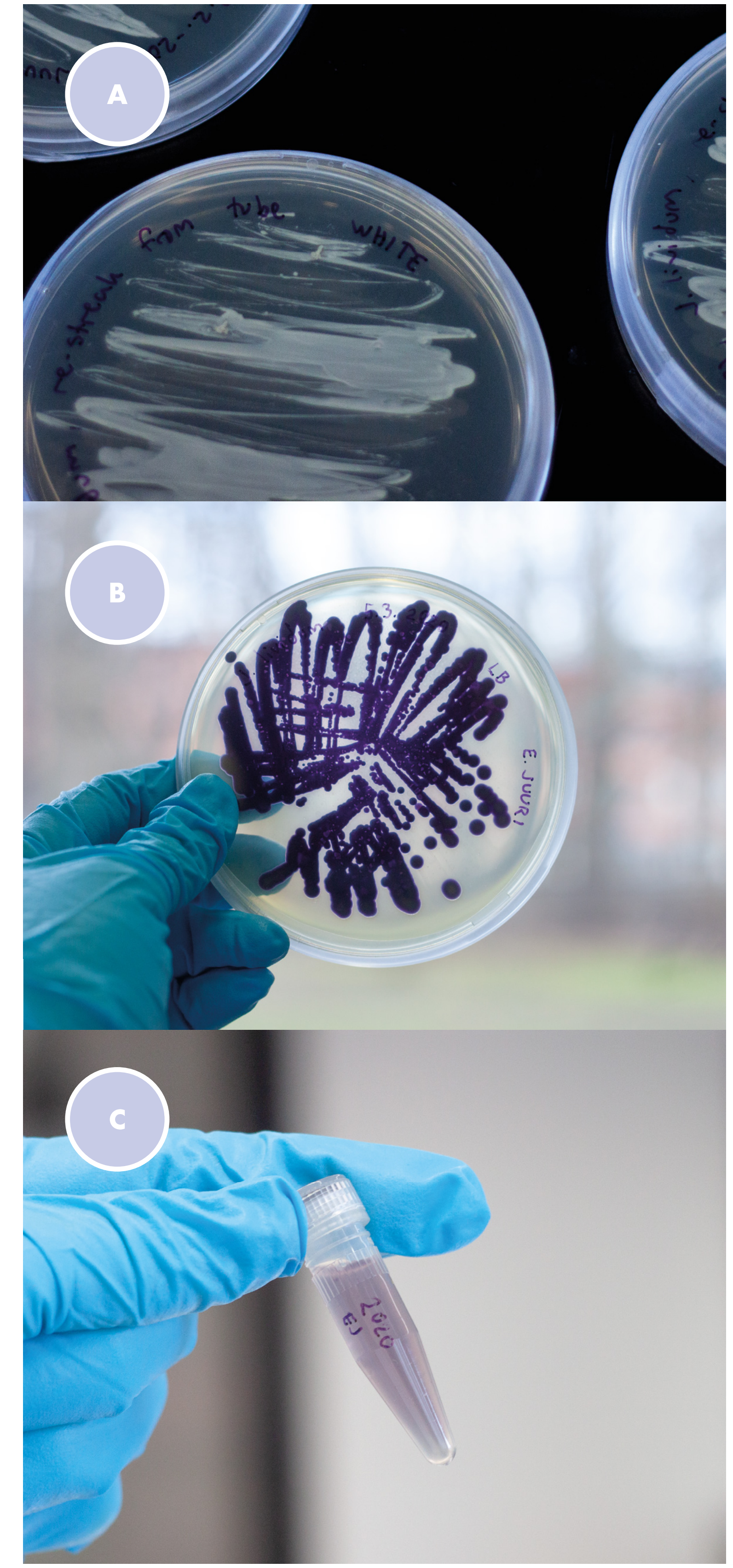
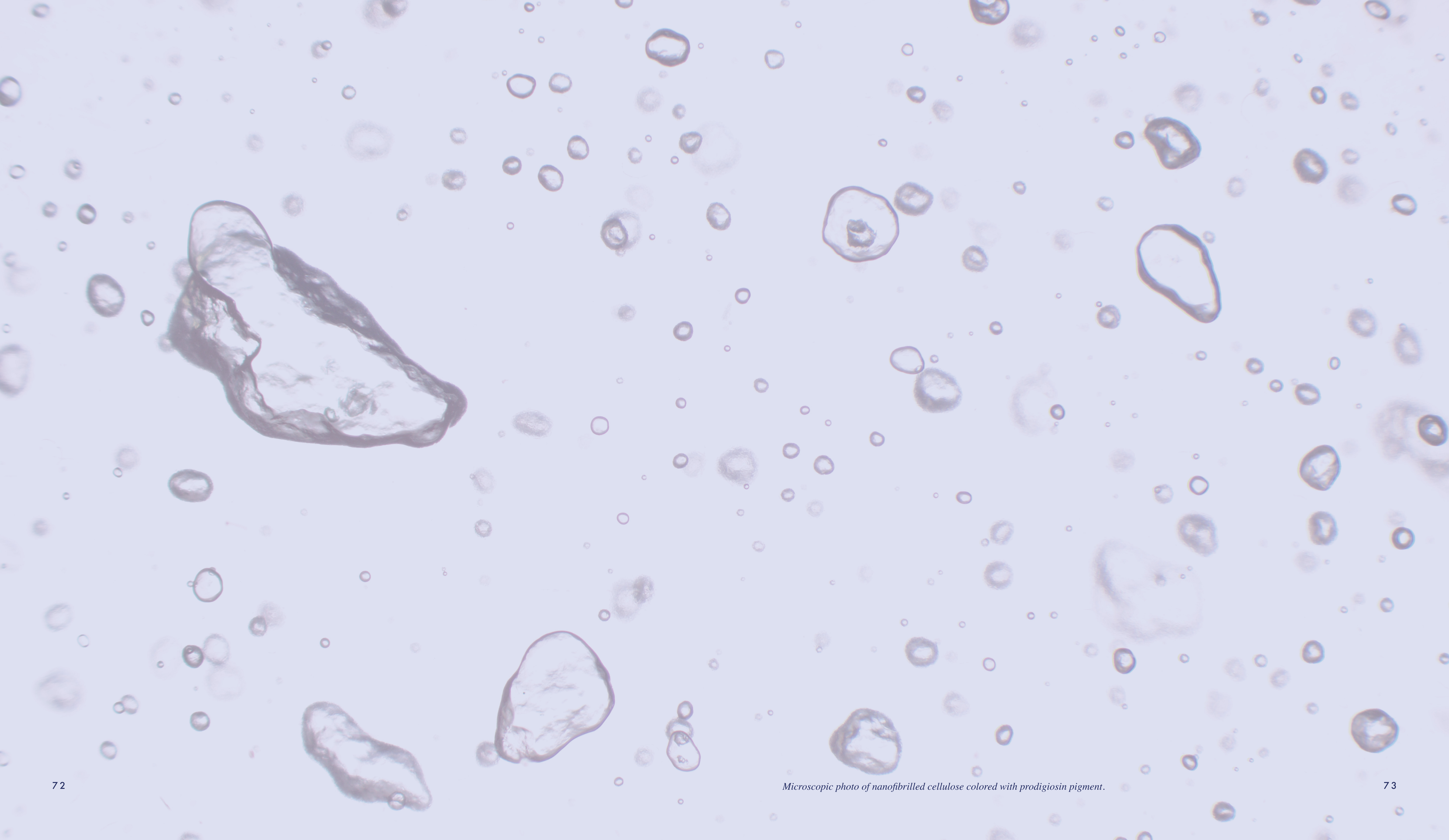


Figure 40:A-C:. Janthinobacterium lividum.



Microscopic photo of nanofibrilled cellulose colored with prodigiosin pigment.

8 DISCUSSION & CONCLUSIONS

This project helped me to understand color and colorants better. Through my studies I have worked with different materials and I have always tried to work with their inherent color because none of the colorant options seemed sustainable enough to me. Especially with bio-based materials it wasn't obvious to me how to color them environment-friendly. Materials and colors walk closely side by side and we shouldn't underestimate the environmental impacts of material coloring. We quite often focus only on the environmental impacts of materials but also color has an impact.

As a result from this thesis project we were able to create colorants and pigments that showed inherent colors of the microorganisms. These colorants were applicable for coloring different materials and showed promising characteristics for future development.

Bacterial colorants and especially prodigiosin pigment created with *S. marcescens* showed as glaring pink color which seems to have huge potential for different applications. Prodigiosin pigment is not water-soluble but solvable in organic solvents and oils. Pigment can also hold multiple health beneficial properties, as mentioned in chapter *4.5 Bacterial Colorants* and the antibacterial properties on fabric have been studied (Ren et al., 2017).

Within this study prodigiosin pigment showed suitability for different purposes and on various materials because of its high concentration, pigmentation and heat-resistance. Bacterial colors can be bright, vivid and saturated which almost remind me of synthetic colorants. Pigment form also enables various coloring methods which can help to reduce water consumption. This gives confirmation that bacterial colorants seemingly have potential to be used for coloring various materials, not only textiles. Bacterial colorants can be produced efficiently in controlled environments, for instance in fermentors. The colorant production doesn't require much water which is a great advantage when compared to commonly used dyes. The color production of prodigiosin with *S.marcescens* happened relatively fast which is a great advantage if the aim is to get this colorant more industrially scaled. In further studies beneficial properties of prodigiosin in different materials should be researched and how pigment effects on the material properties.

Also fungi colorants turned out to be vivid and delicate which can have multiple usage possibilities as well. Some of these colorants were partly water-solvable and some of them were solvable in organic solvents. Fungal colorants showed warmly undertone shades which could be well suited for instance for food coloring.

Figure 41. Fungal colorants.

When comparing bacterial and fungal colorants, color results from these two vary. The characters of the colors had some differences and the nature of the colorants is clearly different: with bacterial colorants colors are relatively bright and intense whereas fungal colorants are more settled and warmly toned. It is brilliant to notice that with two different microorganisms characters of the colorants can be so diverse.

Industries are looking for sustainable alternatives and new innovations are soughts also in the field of colorants. Microorganism produced colorants can offer sustainable alternatives that already are in the market. Scaling of these colorants into industrial use is still in the early stage and it requires years to develop processes that enable color production with microorganisms. We need to build new systems inside of the industries that support the usage of these alternative colorants to be able to compete better with the existing industrially used colorants.

Through this thesis project I realized that it's possible to create colors with microorganisms. It was fascinating to be able to grow your inherent colors with microorganisms and to be able to visually see how the microorganisms create the color. It was interesting to work so closely with living material which made a whole new aspect for the working. Because I started the project without any expectations of the colors, it was enthralling to see what color result we get by letting the microorganisms decide. This really gave you a feeling that we're creating something new.

None of these results wouldn't have been achieved without the help and effort from my mentors. These interdisciplinary projects once again confirmed that there's certainly an advantage when working cross-fields. Multidisciplinary working enables new working tools and expansive new viewpoints that can help to solve design problems. Especially when working color, new types of aspects are more than welcome.

By working with microorganisms provided me also a new viewpoint for color. By fabricating microorganisms as a source for color, the produced color has a different aspect in it where the color is inherent, natural and produced by a living, which again makes it more reasonable. Colors are usually so emotionally charged that we tend to lose the purpose behind the color. By producing color with a microorganism, the color suddenly has a meaning and a story behind it.

Figure 42. Prodigiosin pigment.

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